1-1-1998

Determination of ground-water tracer 2,6-difluorobenzoic acid by Gc/Ms

Cong Han
University of Nevada, Las Vegas

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

DETERMINATION OF GROUND-WATER TRACER 2,6-DIFLUOROBENZOIC ACID BY GC/MS

By

Cong Han

Bachelor of Science
Beijing Institute of Technology
1990

A thesis submitted in partial fulfillment
of the requirement for the degree of

Master of Science

In

Chemistry

Department of Chemistry
University of Nevada, Las Vegas
May 1998

UNLV

Thesis Approval
The Graduate College
University of Nevada, Las Vegas

February 20, 1998

The Thesis prepared by

Cong Han

Entitled

The Determination of Ground Water Tracer: 2,6-Difluorobenzoic Acid By GC/MS

is approved in partial fulfillment of the requirements for the degree of

Master of Science

Examination Committee Chair

Dean of the Graduate College

Examination Committee Member

Examination Committee Member

Graduate College Faculty Representative

ABSTRACT

Determination of Ground-Water Tracer 2,6-Difluorobenzoic Acid by GC/MS

by

Cong Han

Dr. Klaus J. Stetzenbach, Examination Committee Chair
Director of HRC
University of Nevada, Las Vegas

In this study, a GC analytical method for the determination of the ground water tracer, 2,6-difluorobenzoic acid (2,6-DFBA) at the part per billion level was established. Three sample preparation methods, which include two methylation methods and one silylation method, have been evaluated. Chromatographic instruments including GC/MS, GC/ECD, and GC/FID have been used. Silylation of 2,6-DFBA combined with GC/MS analysis has proven to be the best method in this study, due to the low detection limits (part per trillion) achieved, and the stability of the 2,6-DFBA silyl derivative. A GC/MS instrument calibration curve was established, a C-well water sample was analyzed with this method and results were compared with HPLC analysis which has been used to analyze 2,6-DFBA at the part per billion level in ongoing studies.

Since the GC/MS has the ability to separate the silyl derivatives of the various difluorobenzoate isomers, several difluorobenzoates can be analyzed simultaneously by this method in cases where multiple tracers are needed. More work should be done towards achieving better extraction efficiency and reducing the sample preparation time.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

The author would like to express her most special thanks to her advisor Dr. Klaus J. Stetzenbach, the director of HRC, for much appreciated advice, his many times patiently editing her manuscripts, and the financial support.

She is also very grateful to Gary W. Coates, the tracer project manager of HRC, for his knowledgeable guidelines, special effort put through this research, and for his patient reading and editing of her manuscripts.

She would like to thank the committee Dr. Vernon F. Hodge, for his innumerable suggestions and the many changes made to the manuscripts; Dr. Brian J. Johnson, and Dr. David E. James for their valuable advice and crucial time.

The author wishes to express her gratitude to all the members in HRC and Chemistry Department, for their help and support in this project, especially Kaz Linderly, Irene Farnham, Jeannette Daniels, Dr. Spencer Steinberg, Dr. David Emerson, and Dr. Richard L. Titus.

Finally, but not least, she would like to thank her husband Richard Z. Shi, and daughter Patricia Shi, for their constant love and support.

CHAPTER 1

INTRODUCTION

1.1 Purpose of This Study

The purpose of this study was to find a sensitive analytical gas chromatography (GC) method to improve 2,6-difluorobenzoic acid (2,6-DFBA) detection limits to the part per billion (ppb) or part per trillion (ppt) level (high performance liquid chromatography - HPLC detection limit about 3~5ppb) to enhance the use of 2,6-DFBA as ground water tracer. Therefore, gas chromatography/mass spectrometry (GC/MS), gas chromatography with flame ionization detector (GC/FID) and gas chromatography with electron capture detector (GC/ECD) were examined along with several sample preparation procedures. GC/MS was used not only for its better detection limits, but also for the mass spectrometer's capability of positive compound identification. Several derivatization reactions were utilized for improved compound volatility, peak shape, and enhanced detectability (Poole and Schuette, 1984).

Since neither the methyl ester nor the silyl ester of 2,6-DFBA were available commercially, there was no standard to compare with in this study. Therefore, the results are based on a theoretical calculation.

1.2 History of Tracers

Especially in recent years, groundwater pollution due to chemical leaching from point and non-point sources has been of critical concern. Tracers have been widely used to follow the movement of water through soils and aquifers to determine the flow patterns of groundwater and hydrogeologic parameters.

In many aquifer and vadose zone experiments, the availability and use of suitable tracers are essential. An ideal tracer for following the movement of water should be conservative (experiencing neither gain or loss, during transport), nonreactive with the mineral and organic fractions of the solid matrix, not present in the system or with a low background concentration, inexpensive to apply and analyze, and nontoxic (Davis et al, 1980).

While no perfect nonreactive tracer exists, low molecular weight anions (particularly Br^-) approach this ideal since they undergo little interaction with most natural porous media, are environmentally acceptable (most bromide compounds have relatively low toxicities), are readily available, and can be economically analyzed (Bowman, 1984a). However, it would be advantageous if additional tracers were available with similar transport properties, especially when two or more tracers are required for an experiment.

Fluorinated benzoic acid derivatives have many of the properties required of nonreactive soil and ground water tracers (Stetzenbach et al.,1982; Bentley, 1983; McCray et al, 1983; Bowman, 1984a). It was found that several of these fluorobenzoates, such as o-(trifluoromethyl)benzoate (o-TFMBA), 2,6-DFBA, and

pentafluorobenzoic acid (PFBA), behaved almost identically to Br^- under the conditions tested and are acceptable substitutes (Young and Boggs, 1990). These acids, with negative-log dissociation constants (pK_as) less than 4.0, are anionic at typical ground water pHs, are resistant to chemical and microbial transformation, are not known to be toxic to plants and mammals at low concentrations (Stetzenbach and Farnham, 1994), and are readily analyzed at ppb levels in sub-mL water samples using HPLC techniques. Use of the fluorobenzoates is warranted when common inorganic anions such as Br^-, Cl^-, or NO_3^- are not suitable, or when multiple tracers are required.

Fluorobenzoate tracers have been used in studies by several investigators (Bowman and Rice, 1986; Jaynes and Rice, 1993; Hatfield and Stauffer, 1992; Boggs et al., 1992). Detailed evaluation of these chemicals as tracers has, however, been conducted mostly on neutral to alkaline, low organic content ground waters. Suitability of fluorobenzoates as tracers on the more neutral, high organic fraction soils, common in the Midwest, has not been documented, except a single soil sample from North Dakota (Bowman and Gibbens, 1992).

Indication that fluorobenzoates may not be universal surrogates for Br^- was documented in Boggs and Adams (1992), who observed several benzoates retarded differentially to Br^- in an acid sandy material. There is also concern that benzoates may not be suitable in all soils and ground water environments. While toxic to soil microorganisms at high concentration (Seuferer et al., 1979), it has been shown that many of the difluorobenzoates used by Bowan and Gibbens (1992) can be degraded by bacteria in soils and hydrosoils (Cass et al, 1987; Rossiter et al., 1987) with half-lives as

short as 9 to 28 days (Nimmo et al, 1984; Verloop and Ferrell, 1977). That degradation was not observed by Bowman (1984) and Bowman and Gibbens (1992). It may be due to the low organic content, relatively sterile soils used in Bowman and Gibbens's studies.

Fluorinated organics have received less attention compared with chlorinated organics because fewer are regulated, measurement of nonvolatile perfluorinated organics is more difficult, and they are perceived as more inert biologically and therefore less likely to have an impact on human health or the environment (Key et al, 1997). The perception of inertness and its environmental significance are debatable. Inert molecules tend to be persistent and accumulate in the environment, and they are more difficult to remediate. In addition, although these compounds are generally viewed as recalcitrant because of their lack of chemical reactivity, many fluorinated organics are biologically active.

Of the fluorobenzoates which to date have seen extensive field applications as tracers, only the two with aromatic-ring substitution by fluorine, pentafluorobenzoate [PFBA] and 2,6-difluorobenzoate [2,6-DFBA], have shown long-term resistance to chemical and biological breakdown in a variety of hydrologic environments (Bowman,1992). These fluorobenzoic acids, with pK_a less than 4.0, are anionic at ground water pH (calculation is shown below), and are typically analyzed in natural

$$R\text{-}COOH \Leftrightarrow RCOO^- + H^+$$

$$pH = pK_a + \log [\, RCOO^- / R\text{-}COOH \,]$$

Ground water $pH \cong 7$, when $pK_a < 4$

$$\log [\, RCOO^- / R\text{-}COOH \,] > 3;\ [\, RCOO^- / R\text{-}COOH \,] > 1000$$

water samples via HPLC with ultraviolet-visible (UV/VIS) detection (Bowman, 1984b). The procedure requires minimal sample pretreatment and allows accurate measurements of tracer concentrations in the presence of high background levels of Cl^-, NO_3^- and naturally-occurring organic solutes. Detection limits of 2,6-DFBA, PFBA, o-TFMBA, and m-(trifluoromethyl)benzoate (m-TFMBA) were in the range of 1.2 to 2.5 ng, which was based on 5μL (25 ng) injections of a standard solution having a 5ppm concentration of each anion. Retention times were in the 7.3 minutes to 12.5 minutes range. For analysis of anions in soil extracts, practical limits for reliable quantification were about an order of magnitude higher than the previous values.

Recent studies done at the Harry Reid Center for Environmental Studies at University of Nevada, Las Vegas (UNLV) using HPLC with UV detection, have shown detection limits for 2,6-DFBA of 3ppb, quantitation limits of 10ppb, and a linear relationship was achieved in the range of 10ppb to ppm levels.

Ion chromatography (IC) analysis of these fluorobenzoates using conductivity detection is also an accurate and expedient means of determining both single and multiple fluorobenzoate and Br^- concentrations in soil solutions and natural waters (Pearson, et al, 1992). Retention and sample analysis times were found to be less than currently used HPLC methodologies. Mixed and single standard solutions (100ppb – 25ppm) of PFBA, 2,6-DFBA, o-TFMBA, LiBr were prepared and analyzed. High resolution among all four tracers was achieved. Retention times of o-TFMBA, 2,6-DFBA, and PFBA by IC analysis ranged between 1.79 and 2.62 min with a linear detection response achieved across a 250ppb to 25ppm concentration range.

1.3 Gas Chromatography of Organic Acids

Gas chromatography is the technique of choice for the separation of thermally stable volatile organic compounds. However, carboxyl groups, owing to their polarity and a tendency to form hydrogen bonds, are responsible both for the low volatility of the compounds and for other phenomena that make direct GC analysis either difficult or impossible. Carboxyl groups may exhibit strong adsorption on the support of the stationary phase and asymmetry of the peaks. Thermal and chemical instability of the compounds causes losses of the sample compounds in the chromatographic system; i.e., their non-quantitative elution or the elution of decomposition products. Because of these problems, derivatization is usually employed when analyzing carboxylic acids by GC (Drozd, 1981).

1.4 The Use of Chemical Derivatives in Gas Chromatography

Derivatization, e.g., esterification or silylation, in effect a microchemical organic synthesis, is used to convert the protonic functional carboxylic acids to thermally stable non-polar esters or silyl derivatives in order to reduce the polarity of these compounds and thus enhance their chances for successful GC analysis (Poole, 1984). The derivatized compound, by improving the thermal stability and adjusting the volatility of the compound, exhibits shorter retention times, improved peak shape on the chromatogram. Derivatization minimizes undesirable column interactions which could lead to irreversible adsorption and skew peak formation. Therefore, carboxylic acids are analyzed by GC almost exclusively in the form of derivatives.

1.4.1 Types of Carboxylic Acid Derivatives

1.4.1.1 Methyl Esters

Esterification is a typical means of derivatizing carboxyl groups and the esterified form of the carboxyl groups are analyzed by GC to eliminate the interferences encounted in analyzing the original acids. Methyl esters are the most often used as they have a sufficient volatility even for the chromatography of higher fatty acids (Drozd, 1981). Reactions with methanol can be catalyzed with hydrochloric acid, sulfuric acid, boron trifluoride, boron trichloride, or ion-exchange resin. The methanol method is based on the reaction shown below.

$$R\text{-}COOH + CH_3OH \xrightarrow[\Delta]{H^+} R\text{-}COOCH_3 + H_2O$$

The methanol-BF_3 reagent is commonly available and sufficiently reactive even towards strongly hindered groups. A high reactivity, on the other hand, brings about the possibility of undesirable side-reactions if the substrate contains double bonds or other reactive centers. It is reported that methanol-BF_3 gives rise to losses of unsaturated esters and that oleic acid provides a high yield of isomers of methoxymethyl stearate.

The methylation methods have been compared and the results show that methods requiring more complicated procedures give lower values of concentrations. The losses caused by the volatility of methyl esters are most significant with lower molecular weight acids, with unacceptable standard deviations. (Vorbeck, 1961)

1.4.1.2 Silyl Derivatives

The most versatile and universally applicable derivatizing reagents for polar molecules containing protonic functional groups are the alkylsilyl reagents (Poole, 1984). Nearly all functional groups which present a problem in GC can be converted to alkylsilyl ethers or esters. The most common derivatizing reagents are the trimethylsilyl (TMS) reagents. Their derivatives are volatile and generally thermally stable, with good separation characteristics.

The rate of the silylation reaction is affected by the silylating reagents and organic compounds (Poole, 1984). The silylating reagents are ranked according to their "silyl donor ability" and the functional groups of organics are ranked according to their "silyl acceptor ability". For the TMS reagents the approximate order of "silyl donor ability" is: trimethylsilylimidazole (TMSIM) > N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) > N,O-bis-(trimethylsilyl)acetamide (BSA) > N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) > N-trimethylsilyldiethylamine (TMSDEA) > N-Methyl-N-(trimethylsilyl) acetamide (MSTA) > trimethylchlorosilane (TMCS) with base > hexamethyldisilazane (HMDS). For organic functional groups the approximate order of "silyl acceptor ability" is: alcohols > phenols > carboxylic acids > amines > amides. The reaction between a good "silyl donor" and a good " silyl acceptor" is likely to be facile and quantitative under mild conditions.

The rate of the silylation reaction is also affected by steric factors, the use of catalysts, the choice of solvent, and the reaction temperature (Poole, 1984). The silylating reagents themselves have good solubilizing properties for many compounds

and can be used without additional solvent. The primary criterion for selecting a solvent is that it must dissolve both substrate and reagents. Increasing the temperature of the reaction will often improve substrate solubility and enhance the rate of reaction, but it is also possible that the increased temperature will cause the loss of the volatile products. Besides the wide applicability and ease of use of the TMS reagents, the fact that most reactions occur cleanly without artifact or by-product formation adds to the attraction of these reagents.

The mass spectra of TMS derivatives are characterized by weak or absent molecular ions. For difluorobenzoic acid TMS derivatives, the mass spectra have the major peaks at $[M\text{-}CH_3]^+$, $[M\text{-}OSi(CH_3)_3]^+$, $[M\text{-}COOSi(CH_3)_3]^+$, and $[Si(CH_3)_3]^+$, which correspond to the m/e ratios 215, 141, 113, and 73 respectively (Wu, 1996). Ions formed by cleavage of a methyl to silicon bond, m/e 215 are generally more abundant. This ion can be used to determine the molecular weight provided that is not mistaken for the molecular ion itself. Dissociation of the molecular ion often results in prominent secondary fragment ions containing the ionized dimethylsiloxy group attached to a hydrocarbon portion of the molecule. In common with alkyl ethers, cleavage of the bond adjacent to oxygen is favored, the m/e 73 ion is prominent in virtually all TMS spectra.

Among the less desirable features of the trimethylsilyl derivatives are their limited hydrolytic stability and poor intrinsic detection characteristics for trace analysis, and, in addition, the trimethylsilyl group shows no particular electron-capture properties (Poole, et al, 1980), the response of the electron capture detector towards halgen-containing compounds follows the order I > Br > Cl > F. On occasion, higher alkyl homologs or halogen-containing alkyl or aryl substituted analogs of the TMS derivatives are used to impart greater derivative hydrolytic stability, improved separation characteristics, increased sensitivity when used with selective detectors, or to provide mass spectra containing greater diagnostic information (Poole and Zlatkis, 1979).

A method has been described for the preparation of silyl derivatives even in the presence of water. Its principle is in the addition of such a large excess of the silylating agent that all of the water is removed (Weiss and Tambawala, 1972). The extent to which the presence of water affects the reaction yield and whether or not a large excess of by-products has an adverse effect must be tested. Because of the possible sensitivity of the derivatives towards moisture, they should be prepared immediately prior to the analysis, even though they have been reported to be stable under anhydrous conditions for a few days (Horning and Boucher, 1968).

1.5 INSTRUMENTATION

1.5.1 Gas Chromatography

GC as a method of instrumental analysis is capable of producing information which may describe the qualitative and quantitative composition of mixtures of

compounds. In GC, a gaseous transport medium (mobile phase) is applied for the separation of the sample components after they have been introduced into the chromatographic system. The two important elementary parts of the chromatographic system are the column, in which the separation takes place, and the detector.

Chromatographic separations are based on multiple partition of the compounds to be separated between two phases. The mobile phase (carrier gas) carries the gaseous sample aliquot through a column containing a stationary phase. The individual components (solutes) in the sample aliquot are temporarily dissolved in the liquid stationary phase (silicone polymers) or adsorbed on the surface of the solid stationary phase at different rates resulting in their separation. The separated sample components then enter the detector, and the signals produced in the detector are proportional to the concentration of the separated species. The chromatogram (a graph of the detector signal) and a report which contains figures for the retention times and peak areas (also peak heights) of all or of selected peaks are produced for each mixture introduced into the column.

1.5.1.1 FID

Flame ionization detector is by far the most popular detector for gas chromatographic effluents. These detectors operate on the principle that the electrical conductivity of a gas is directly proportional to the concentration of charged particles within the gas. In the FID a hydrogen flame serves as the ionizing source. Carrier gas moves the sample components from the column into the flame, which ionizes some of the

organic molecules in the gas stream. The presence of the charged particles (positive ions, negative ions, and electrons) within the electrode gap causes a current to flow through a measuring resistor. The resulting voltage drop is amplified by an electrometer and fed to a recorder. The mechanism for the production of ion current can be simply explained by the fact that the free radicals $CH_3^{\cdot}$, $CH_2^{\cdot}$, $CH^{\cdot}$ are the result of pyrolytic reactions of the organic compounds. When the free radicals encounter either oxygen atoms or high-energy oxygen molecules, a series of ion-molecule reactions take place, producing positive ions (Bruner, 1993).

$$CH + O^* \rightarrow CHO^+ + e$$

$$CHO^+ + H_2O \rightarrow H_3O^+ + e + CO$$

A schematic view of a typical FID is shown in Figure 1.1.

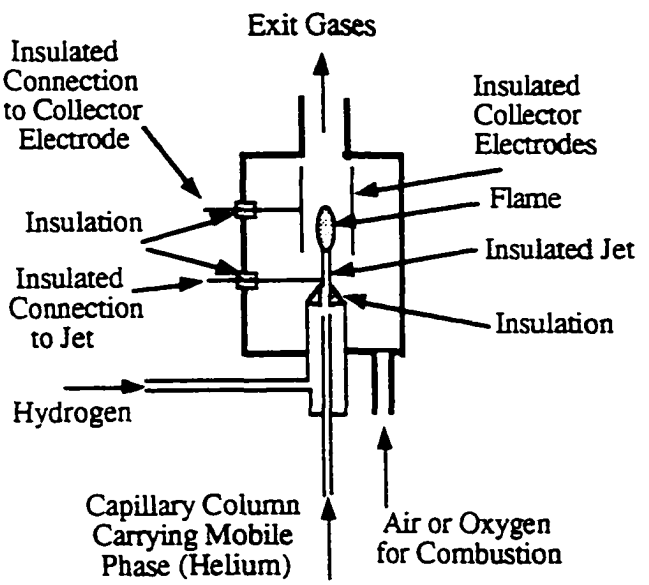


Figure 1.1 The Flame Ionization Detector (Techniques and Practice of Chromatography, New York, 1995).

The great success of the FID is due to the following characteristics (Bruner, 1993):

* The ion current is extremely low when pure hydrogen is burned in a flame fed by pure air in the absence of any organic compounds, this implies a negligible baseline background.
* A very large number of ions is produced if an organic compound burns in the flame compared to the background produced by the hydrogen.
* The linear dynamic range of FID is about 1×10^{7}.
* The sensitivity of FID toward hydrocarbons, either aliphatic or aromatic, is very large, signals can be obtained at picogram level.

1.5.1.2 ECD

The selective electron-capture detector is the second most widely used detector (Poole, 1984). The ECD measures the loss of signal rather than an increase in electrical current. As the carrier gas flows through the detector, radioactive ^{63}Ni ionizes the gas and thermal electrons are formed. These electrons migrate to the anode, which normally has a potential of ca. 90V. When collected, these electrons produce a standing current of ca. 10^{-8} A, which is amplified by an electrometer. If an electron-capturing compound enters the detector it captures a thermal electron to produce either a negative ion or a fragment ion if dissociation accompanies capture, as shown below.

$$AB + e^{\cdot} \rightarrow AB^{\cdot}$$

$$AB + e^{\cdot} \rightarrow A^{\cdot} - B^{\cdot}$$

$$AB + e^{\cdot} \rightarrow A^{\cdot} - B^{\cdot}$$

The operating conditions are optimized such that the thermal electrons are collected but the negative ions are not. The diminution in detector background current due to the loss of thermal electrons constitutes the quantitative basis by which detector response is related to solute concentration. A diagram of an electron capture detector is shown in Figure 1.2.

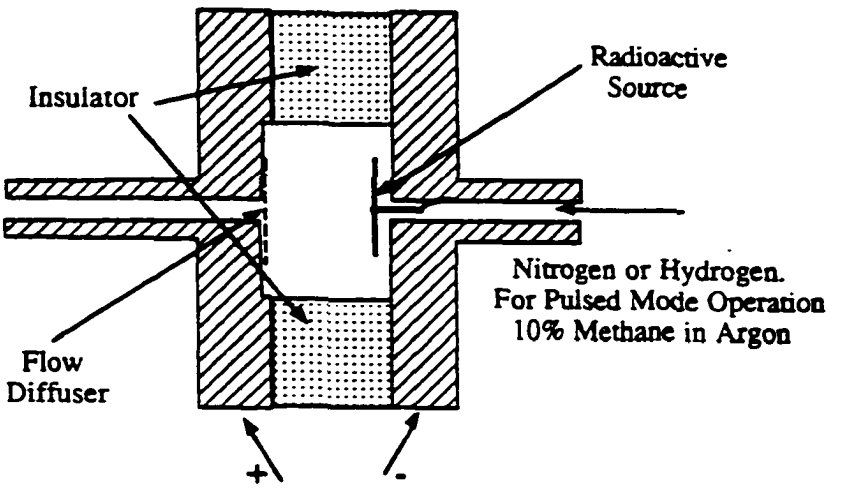


Figure 1.2 The Electron Capture Detector(Techniques and Practice of Chromatography, New York, 1995)

ECD is extremely sensitive to molecules containing electronegative atoms or groups, which easily capture an electron, such as polyhaloalkanes, conjugated carbonyls, nitriles, nitrates, aromatic polynitro compounds, and organometallics. Responses towards the halogens decrease in the order I > Br > Cl > F. The ECD is virtually insensitive to hydrocarbons, alcohols, and ketones. ECD has a very limited linear

dynamic range, and is very sensitive to changes in carrier gas flow rate and temperature (Bruner, 1993).

1.5.1.3 Mass Spectrometer

The mass spectrometer detector has the ability to separate gaseous positive ions according to their mass-to-charge ratio (m/e). The basic components of a mass spectrometer are shown schematically in Figure 1.3. Under normal operating conditions, the vacuum systems associated with the

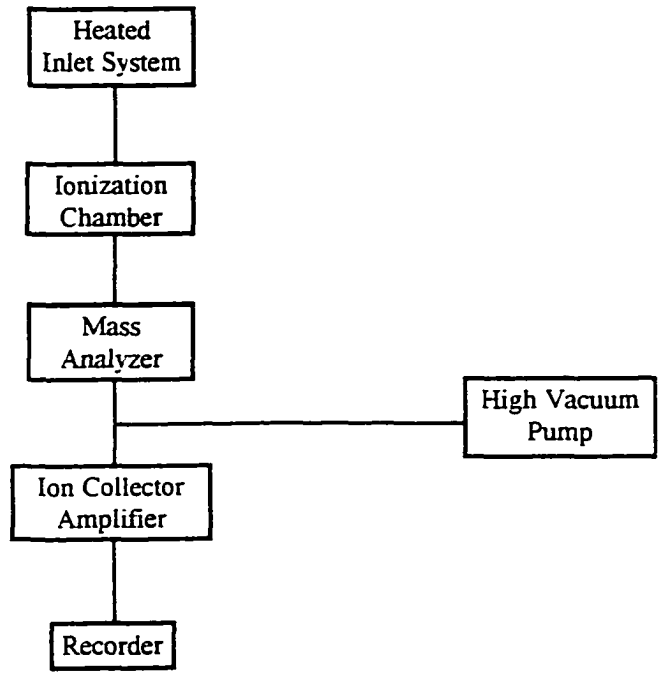


Figure 1.3 Basic components of a mass spectrometer.

instrument maintain it at the low pressures required to avoid intermolecular and interionic reactions. Whether the compound is a gas or a volatilized liquid, the heated

inlet system allows the material to enter the highly evacuated ionization chamber through a molecular leak whose conductance is preferably in the 0.1 to 0.4 cc/sec range. The gaseous molecule is bombarded in the ionization chamber and the positive ion fragments (which are more abundant than the negative ions by several orders of magnitude) are accelerated from the ion source electrostatically and then resolved or separated according to their mass-to-charge ratio by the mass analyzer. These mass separated ions of a particular m/e impinge sequentially on an ion collector electrode, causing an electrical current which is amplified 10^3 to 10^8 times with virtually no noise into an electrical signal that is proportional to ion abundance and compatible with fast recording devices (Gudzinowicz, 1976). The advantage of a MS as a GC detector over the FID or ECD is that it has better qualitative capabilities allowing a more positive identification of sample components.

1.5.1.3.1 Electron Impact Ionization

Electron impact ionization is the most common ionization method used with organic compounds (Biemann, 1962). Ion formation from sample molecules in the gas phase is based on an exchange of energy during collisions between energetic electrons and neutral gas molecules or atoms, which produces a molecular ion, an odd-electron ion usually in a high state of electronic and vibrational excitation. The relative amounts and type of ions formed depend on the sample's chemical nature and the bombarding electron energy, which is nearly 70eV for most organic substances. Fragmentation as well as

dissociative and multiple ionization occur, the resulting mass spectrum thus becoming a "fingerprint pattern" of the particular compound under study.

1.5.2 High Performance Liquid Chromatography

Only about 20% of known compounds can be analyzed by GC without prior treatment (Meyer, 1988). The other 80% are insufficiently volatile or can't pass through the column or because they are thermally unstable and decompose under the conditions of separation. HPLC is not limited by sample volatility or thermal stability. HPLC is able to separate macromolecules and ionic species, labile natural products, polymeric materials, and a wide variety of other high-molecular weight polyfunctional groups (Willard and Merritt, 1988). A diagram of a suitable instrument for HPLC is shown in Figure 1.4.

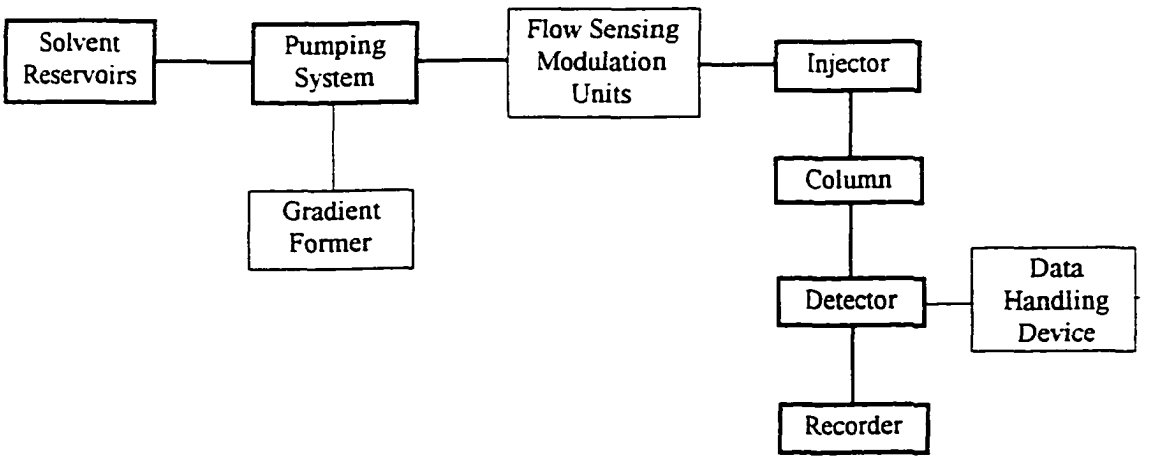


Figure 1.4 The diagram of HPLC.

For isocratic elution, a solvent is unchanged throughout the time and delivered on to the column. In gradient elution two or more solvents are mixed in proportion so that the concentration of the stronger solvent increases with time. Between pump and injector there may be a series of devices which ensure a homogeneous, pulse-free liquid. Flow is delivered to the column at a known pressure and volume flow rate. An injection device is connected to the head of the column for loading the sample. A detector is connected to the end of the column which produces signals that are proportional to the concentration of the separated species. A chromatogram and report much like those produced from a GC can be produced by the data system on the HPLC.

1.5.2.1 Reversed-Phase System

In reversed-phase systems, the mobile phase is more polar than the stationary phase. Reversed-phase systems with a chemically-bonded stationary phase are very widely used in chromatography. Compared with chromatography with a liquid stationary phase, this method has much higher reproducibility, stability, separation efficiency and the possibility of employing gradient elution. Thus, chromatography on non-polar chemically bonded phases has practically replaced liquid-liquid chromatography. Various types of substances can be chromatographed, ranging from non-polar hydrocarbons to completely ionized sulphonic acids and small inorganic ions, including biopolymers and other macromolecular substances.

Chromatography in reversed-phase systems employs mobile phases containing water and one or more organic solvents that are miscible with water, usually methanol, acetonitrile, tetrahydrofuran, dioxane, propanol, etc.(Locke, 1974).

In the chromatography of strongly polar or ionized solutes it is necessary to add buffers, salts, or reagents that form ionic associates, to the mobile phase. Strongly polar and ionic substances are usually retained very slightly or not at all in the reversed-phase system and, as a result of ionic exclusion, some are eluted sooner than in a time corresponding to the dead volume of the column (volume of the mobile phase in the column). Addition of a suitable buffer to the mobile phase adjusts the pH to suppress dissociation of weak acids at pH<7) and they are usually not difficult to separate in the undissociated form(Melander and Horvath, 1980). The content of organic solvent in the mobile phase also greatly affects the retention. A marked decrease in the solute retention with increasing concentration of organic solvent is usually observed in binary mobile phase.

Gradient elution is also widely used in HPLC, in this case, the composition of the mobile phase gradually changes with time according to a program so that the elution strength of the mobile phase gradually increases. This enables weakly sorbed substances to be eluted by a mobile phase with a low elution strength with good separation, while the elution of substances with strong affinity for the stationary phase is accelerated by increasing the elution strength of the mobile phase in the final stages of gradient elution (Jandera and Churacek, 1985).

1.5.2.2 Detector

The UV-visible spectrophotometer is the most widely used detector for HPLC as it can be rather sensitive, has a wide linear range, is relatively unaffected by temperature fluctuations, and is also suitable for gradient elution (Meyer, 1993). The basis of UV-VIS detection is the difference in the absorbance of light by the analyte and the solvent. A number of functional groups absorb strongly in the ultroviolet, including aromatic compounds, carbonyl compounds. Solvents that absorb only weakly in the UV range include water, methanol, and acetonitrile making them good choices for the mobile phase.

CHAPTER 2

EXPERIMENTAL

2.1 Acidified Methanol Methylation

2.1.1 Chemical Reaction and Methylation Procedure

$$R\text{-}COOH + CH_3OH \xrightarrow[\Delta]{H^+} R\text{-}COOCH_3 + H_2O$$

In this method, the 2,6-DFBA methyl ester was prepared by reacting 2,6-DFBA with acidified methanol (Steinberg, 1996). Methanol was first acidified by adding 3ml of acetyl chloride into 50ml erlenmeyer flask of methanol. Milligram quantities of 2,6-DFBA (18~20mg) were added to 5ml of the reagent in a 20ml screw-capped test tube. The test tube was capped and placed into a VWR Scientific heat block for 1 hour at 100°C. Then, 1 ml of deionized water was added to the mixture and the organic phase was evaporated under a vacuum until the derivative solution was completely dry. Then the dried sample was added with 10 mL methylene chloride. The prepared 2,6-DFBA methyl ester solution was ready for analysis.

2.1.2 Reagents

2,6-DFBA was obtained from Aldrich Chemical Company (Milwaukee, WI).

Chemical purity was 98% according to the manufacturer. The compound was used without further purification. The water used for the sample preparation was obtained by passing deionized water through a Barnstead (Dubuque, IA) Nanopure water system and subsequently distilling the Nanopure water in an all-glass still. (Stetzenbach, 1994). High purity methanol and methylene chloride were both obtained from Burdick & Jackson (Muskegon, MI). Acetyl chloride (98.5%) was A.C.S. reagent obtained from Aldrich (Milwaukee, WI).

2.1.3 GC Instrument Analysis

One microliter of the prepared 2,6-DFBA methyl ester was analyzed on GC/FID for the initial analysis. GC/FID was Hewlett Packard 5890A, the DB-5 GC capillary column was obtained from J&W Scientific (Folsom, CA), 30m × 0.25mm I.D. and 0.25μm film thickness, stationary phase was bonded with (5%-phenyl) methylpolysiloxane. The typical instrument parameters are found in Table 2.1.

Table 2.1 GC Parameters

Column: DB-5	
Temperature	
Oven temp. program:	Initial: 50°C for 4min
	Rate: 50°C to 280°C at 10 C/min
	Final: 280°C for 15min
Injector B: 280°C	
Detector: 280°C	
Flow rate	
Column flow: 1ml/min	
Make up gas He: 35ml/min	
Air: 400ml/min	

The chromatogram showed two peaks yield on GC column at 8.5 minutes and 11.1 minutes (Figure 2.1).

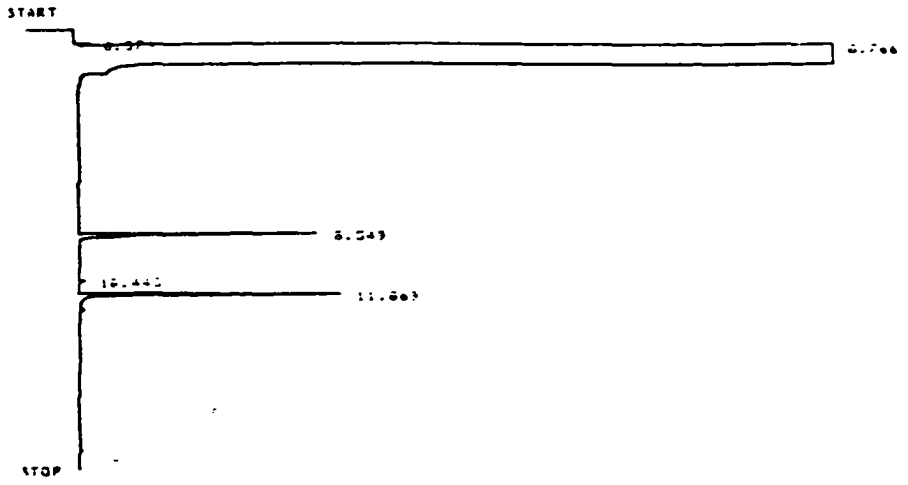


Figure 2.1 Chromatogram of GC/FID analysis of ppm 2,6-DFBA and 2,6-DFBA methyl ester by using the acidified methanol.

To positively confirm these two peaks, one microliter of the methyl ester solution was injected onto the GC/MS column. The GC/MS was a Hewlett Packard (Avondale, PA) 5890 equipped with Electron Impact Ionization ion mode. Typical instrument parameters are found in Table 2.2.

The GC/MS chromatogram yielded two major peaks at retention time 12.3 minutes and 14.6 minutes (Figure 2.2). The peak at 12.3 minute is 2,6-DFBA methyl ester, which can be determined by its characteristic m/e ratios 113, 141, 172. The peak at retention time 14.6 minutes is the free 2,6-DFBA with the characteristic m/e ratio 113,

141, and 158. The small amount of 2,6-DFBA methyl ester produced compared with the large amount of free 2,6-DFBA retained indicated that the reaction efficiency was low.

Table 2.2. GC/MS Parameters

Column: SPB-5	
Oven temp:	Initial: 50°C for 0min
	Rate: 0°C to 280°C at 10°C/min
	Final: 280°C for 0min
Injector: 220°C	
Transfer line: 280°C	
Carrier gas (Helium): 25-30ml/min	
Mass spectrometer	
Ionization: EI	
Scan range: 50-360	
Scan rate: 1.5 sec	

After the GC/MS confirmation of these two peaks, it can be assumed that the peaks in previous GC/FID analysis (Figure 2.1) were 2,6-DFBA methyl ester with retention at 8.5 minutes, and free acid with retention at 11.1 minutes. However, the large amount of 2,6-DFBA means the methylation reaction did not go to completion. This conclusion is based on the assumption that the free acid and methyl ester have nearly the same instrument response. Therefore, the methylation reaction times were prolonged to 3 hours, 4 hours, 5 hours, 7 hours, and 9 hours. The chromatograms are shown in Appendix Figures 1-3. These experiments showed that the methylation reaction did go further when longer times were used. In addition, the loss of the methyl

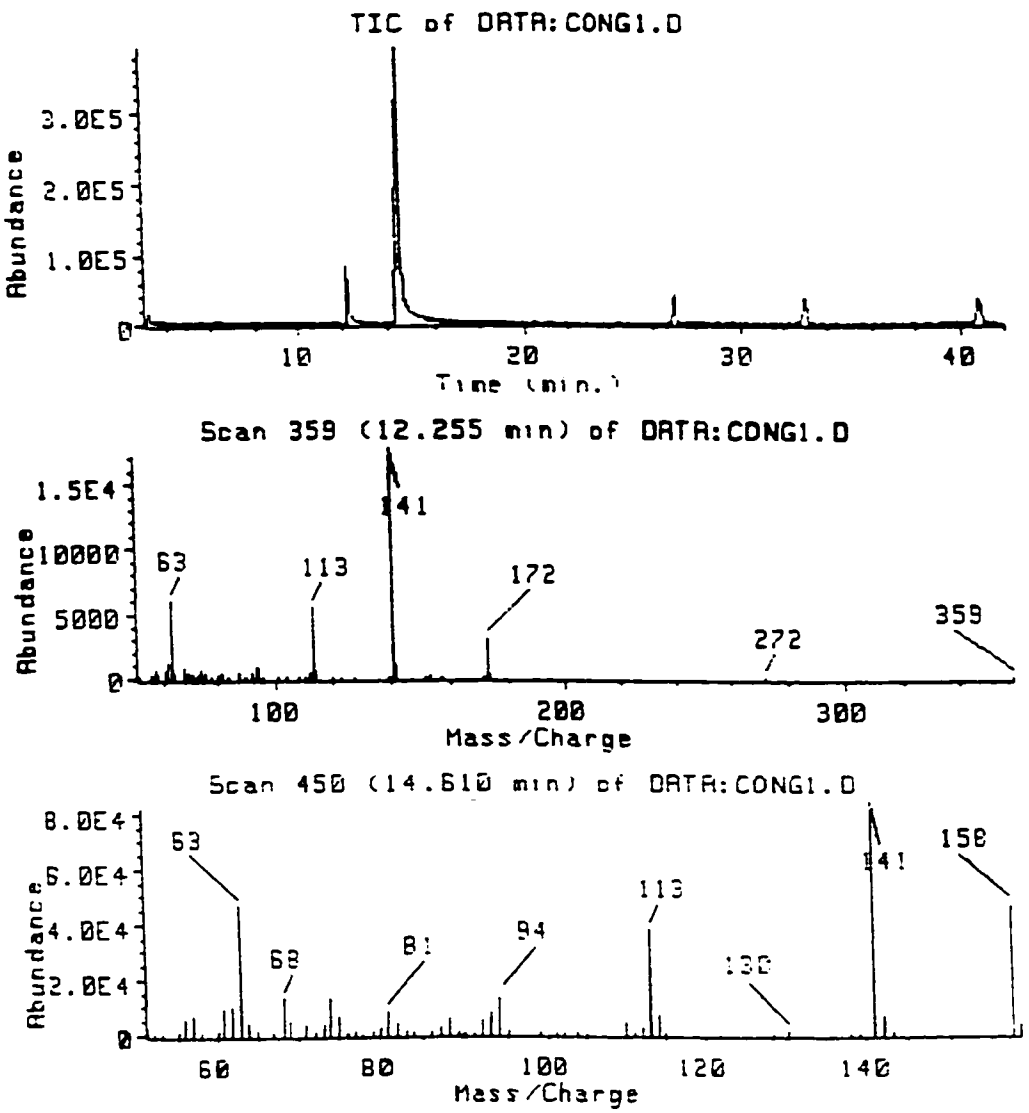


Figure 2.2 Chromatogram of GC/MS analysis of ppm 2,6-DFBA and 2,6-DFBA methyl ester.

ester peak on the chromatogram after 3, 5, and 7 hours reaction indicates this method is not reproducible, this might be due to the loss of the volatile 2,6-DFBA methyl ester during the evaporation step.

Numerous experiments were repeated. The high ppm sample injection only gave about 40,000~60,000 area count on the GC column. The poor sensitivity of the analyte on the GC/FID might be because either the reaction is poor, or due to the large amount lost of the analyte during the methylation reaction and evaporation step.

In conclusion, this methylation method is not efficient and useful due to the long reaction time, poor instrument sensitivity, and non-reproducible result.

Therefore, the BF_3-methanol methylation method was then studied.

2.2 BF_3-Methanol Methylation

2.2.1 Chemical Reaction and Methylation Procedure

$$R\text{-}COOH + CH_3OH \xrightarrow[\Delta]{BF_3} R\text{-}COOCH_3 + H_2O$$

This method uses BF_3-methanol as the methylation reagent instead of the acidified methanol. 2,6-DFBA (up to 25mg in 2ml high purity hexane) was added to 2ml of the 14% BF_3 -methanol reagent in a 10ml test tube. The uncapped test tube was placed in a small beaker of water, and boiled on a steam bath for 3 minutes. 1ml of distilled water was added to stop the reaction. The mixture was cloudy, but after standing for 15 minutes it separated into two phases. The upper layer contained the methyl ester in hexane and the bottom layer was methanol, water, and the acid catalyst

BF_3. A 30 ml separatory funnel was used to separate the two layers. The bottom layer was discarded and the top layer, containing the methyl esters, was dried under high pure nitrogen and diluted with hexane for analysis.

This method was chosen because of its fast methylation reaction compared with the acidified methanol method, only 3 minutes.

2.2.2 Reagents

2,6-DFBA and ionized water were obtained in the same way as in section 2.1.2. Boron trifluoride/methanol (14%) was obtained from Supelco (Bellefonte, PA). Hexane was GC/GC-MS grade from Burdick & Jackson (Muskegon, MI).

2.2.3 Instrument Analysis

One microliter volume of the diluted ppm methyl ester solution was analyzed on HP 5890A GC/ECD equipped with a Supelco SPB™-5 fused silica GC capillary column. The column stationary phase was bonded with poly (5%-diphenyl-95%-dimethylsiloxane). The column was 30m long with 0.32mm internal diameter and 1.0μm film thickness, the column temperature limit range was from -60°C to 320°C. Table 2.3 shows the general parameter of the instrument. No peaks were detected on the chromatogram. The reason might be because of the loss of the methyl ester with the evaporated reagent. Therefore, the heating temperature was reduced from 100°C to 60~65°C, and a 100 mm coil length reflux condensor was equipped to the evaporator appratus to allow additional fluid to be fed to bath during long evaporations to minimize the loss.

Table 2.3 GC Parameters

Column: SPB-5	
Temperature	
Oven temp. program:	Initial: 70°C for 2min.
	Rate: 70°C to 160°C at 6°C/min
	Final: 160°C for 10min.
Injector B: 250°C	
ECD: 280°C	
Flow rate	
Carrier gas (high purity helium): 1ml/min	
Make up gas (nitrogen) + He: 30ml/min	

Another reason might be that the loss of the peak was due to 2,6-DFBA's poor solubility in hexane. In this situation, HPLC analyses were performed to analyze the aqueous phase of the solution after the two layers were separated. Table 2.4 shows the general parameters of the HPLC. The HPLC consisted of a Model SP 8800 pump, Model SP 8880 auto sampler and Model UV/Vis 1000 detector, all by Spectra-Physics (San Jose, CA). The mobile phase was 0.01M potassium biphosphate (KH_2PO_4) buffer, pH adjusted to 2.5 with phosphoric acid, with 25% methanol as an organic modifier. The HPLC was calibrated before running the samples. The calibration standards were 200 ppb, 400 ppb, and 1000 ppb 2,6-DFBA in DI water. The HPLC result showed that 96% of the original amount of the 2,6-DFBA was left over, only 4% of it reacted with BF_3-methanol. This result indicated that hexane is not the suitable solvent for 2,6-DFBA.

Table 2.4 HPLC Parameters

Column: LC-18
Pump: SP 8800 ternary HPLC
Pressure: 1410Psi
Flow rate: 1.5ml/min
Run time: 10min
Injection volume: 50µl
UV/Vis detector: 230nm
Auto sampler: SP 8880
Vial size: 2.0ml
Solvent: 75% KH_2PO_4 (0.01M) @pH2.5
25% Methanol

The methylation procedure was modified to use ethyl acetate as solvent instead of hexane. The GC column was changed to DB-5 column. Table 2.5 shows the GC conditions.

Table 2.5 GC Parameters

Column: DB-5
Temperature:
Oven temp. program: Initial: 35°C for 5min.
Rate: 35°C to 120°C at 3°C/min
Rate: 120°C to 260°C at 10°C/min
Final: 260°C for 0min
Inlet B: 200°C
ECD: 250°C
Flow rate:
Carrier gas (high purity helium): 1.1ml/min
Make up gas (nitrogen) + He: 30ml/min

Methyl esters of 2,6-DFBA were prepared by using the same procedure and analyzed by GC. Only ppm level (the ppm is based on the original amount of 2,6-DFBA in the final diluted solution) peaks were obtained. HPLC was used again to find the reason which caused this low sensitivity.

In HPLC analysis, a calibration curve was generated from 0 to 1000ppb of 2,6-DFBA standards in DI water (Figure 2.3). Methylation reactions were stopped after 1 minute, 2 minutes, 3 minutes, 4 minutes, and 5 minutes separately. The aqueous solutions were diluted with DI water into a 1ppm solution (assuming the whole amount of 2,6-DFBA was in this solution and the concentration was 1ppm) and were then

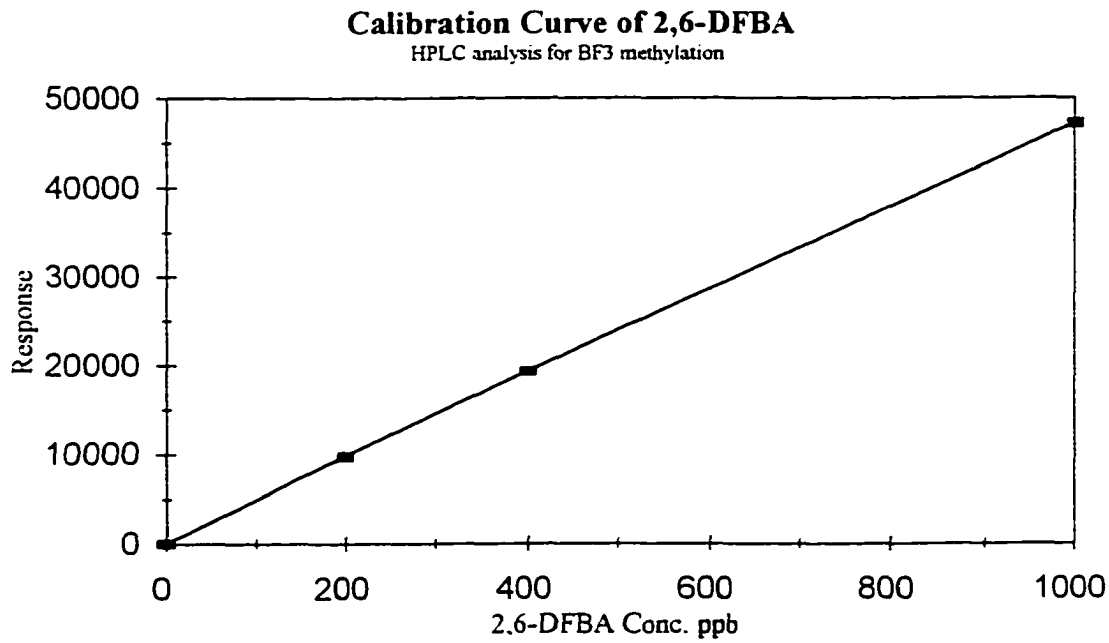


Figure 2.3 Calibration curve of 2,6-DFBA analysis on HPLC for BF_3 methylating method.
Response = X Coef.*Conc
X Coef. = 47.38±0.25, Corr. Coeff = 0.9998

analyzed on HPLC. Chromatograms of the HPLC analyses are shown in Appendix Figure 4-11.

Table 2.6 Methylating reaction efficiency test of 2,6-DFBA by using BF_3-methanol reagent as a function of reaction time.

REACTION TIME MIN	RESPONSE OF 2,6-DFBA	CONC. OF NON-REACTED 2,6-DFBA, PPB
1	1,909	40.3
2	2,033	42.9
3	2,663	56.2
4	2,971	62.7
5	3,334	70.4

The result (Table 2.6) showed that only 4 to 7 percent (40~70ppb/1000ppb) of the original amount of 2,6-DFBA were left over after the reaction, over 93% of the 2,6-DFBA was in the organic phase and reacted with the BF_3-methanol. This indicates 3 minutes is enough for the reaction, and ethyl acetate is a good solvent for 2,6-DFBA.

The large amount of 2,6-DFBA used in the methylation reaction should have yielded a huge peak on the GC/ECD, however, the response was still very low. This low sensitivity of the GC/ECD might be due to a big loss of the analyte during the reaction, or the methylation reaction efficiency is still poor.

In conclusion, BF_3-methanol methylation is still an unacceptable method.

2.3 Silylation Method

2.3.1 Chemical Reaction and Silylation Procedure

Silylation is the most commonly used derivatization technique in the GC of non-volatile substances. The trimethylsilyl derivative of 2,6-DFBA can be prepared by the reaction of the trimethylsilylating agent BSTFA with 2,6-DFBA.

To prepare the silyl derivative, 20ppb to 200ppb of 2,6-DFBA standard solutions were prepared in deionized water. 20ml of each standard solution was pipetted into a 40ml vial. The solution was acidified with phosphoric acid to a pH of 1.9-2.3. Then 5ml of ethyl acetate (dried by molecular sieve before use) was added to extract the 2,6-DFBA out of the water (The reason for the organic extraction is the evaporation of the water is much slower than the evaporation of the organic solvent). A teflon-lined cap was screwed on the vial. The solution was shaken for 60 minutes on a shaker and then allowed to settle for 5 minutes. The organic layer was transferred into a 10ml vial with a glass pipet. The solvent was removed with a gentle stream of nitrogen. 100µl of BSTFA was added and the cap was placed on tightly. The solution was kept at room temperature for 1 hour. 2µl of 100ppm ethyl benzoate was added as the internal standard. The solution was ready for GC/MS analysis.

2.3.2 Reagents

BSTFA was derivatization grade obtained from Aldrich (Milwaukee,WI) with 99+% purity. Ethyl acetate was A.C.S. spectrophotometric grade with 99.5+% purity,

obtained from Aldrich (Milwaukee,WI). Phosphoric acid (12M) was obtained from J.T. Baker (Phillipsburg,NJ).

2.3.3 Instrument Analysis

Two microliters of the reaction solution potentially containing 2,6-DFBA trimethylsily (TMS) derivative was first analyzed on GC/MS for positive identification of the derivative. The typical instrument parameters are found in Table 2.7. The GC/MS

Table 2.7 GC/MS Parameters

GC Method: DFBATMS1
- Column: DB™-5 ms
- Oven temp programming:
  - Initial: 75°C for 1 min
  - Rate1: 75°C to 140°C at 10°C/min
  - Rate2: 140°C to 240°C at 50°C/min
  - Final: 240°C for 0.5 min
- Injector: 220°C
- Transfer line: 290°C

Aquisition Method: DFBATMS1
- Mass range: 100 to 250 m/z
- Scan time: 1.500 sec
- Segment length: 9.00 min
- Fil/Mul delay: 3.00 min
- Peak threhold: 1 count
- Mass default: 0 mu/100mu
- Background mass: 69 m/z

Ion mode: EI
Ion preparation: None
Ion control: Auto
Carrier gas: High Purity Helium

used was a Varian 3400 GC with a Saturn 2000 mass spectrometer. The data were recorded with Varian Saturn computing software. The analytical column was a J&W Scientific 30m × 0.25μm i.d. DB™-5 ms GC capillary column, bonded with (5%-phenyl)-methylpolysiloxane.

After the identification of the 2,6-DFBA TMS by GC/MS, more experiments were performed to determine the GC/MS instrument detection limits, the silyl derivative stability, and the GC/MS instrument calibration curves were generated, the C-Well field sample was analyzed as well.

2.3.3.1 The Test of the Silylation Reaction

In this study, the silylation reaction mixture was not heated as it had been done by Wu (1996), the purpose was to avoid any possible loss of the product during the heating process. Since Wu was using the ppm level of the 2,6-DFBA to prepare the 2,6-DFBA TMS, and then qualitatively determining the analyte on GC/MS, the loss of the volatile analyte did not hurt the positive determination of the compound. However, in this study, the 2,6-DFBA TMS solutions were made from the ppb levels of 2,6-DFBA standards. Any small amount of loss during the heating might adversely affect the quantitative determination that was desired.

The necessary time for the complete silylation reaction was tested to determine if one hour was long enough. Two microliter volumes of the 2,6-DFBA TMS derivative solutions, made from the 20ppb to 200ppb of 2,6-DFBA standards, were injected on to the GC/MS column after 1 hour and 24 hours reaction times.

2.3.3.2 The Detection Limits Tests

Two microliter volumes of a 2,6-DFBA TMS derivative solution, which made from a 5ppb 2,6-DFBA standard, was used to perform this test. Seven portions of this solution were analyzed right after the silylation reaction and the addition of the internal standard. Two detection limits, method detection limit and quantitation detection limit were calculated.

2.3.3.3 The 2,6-DFBA TMS Stability Test

Since the 2,6-DFBA TMS derivative is very sensitive to moisture, it was assumed that the derivative should be analyzed immediately after the preparation (Poole, et al, 1980). A one to 24 hours stability test was performed. The 2,6-DFBA TMS solutions prepared from 40ppb, 100ppb, and 200ppb of 2,6-DFBA standards were analyzed after 1, 2, 3, 4, 6, 9, 24 hours on the GC/MS. The responses were calculated and compared.

2.3.3.4 GC/MS Instrument Calibration Analysis

In order to analyze the C-Well sample, the GC/MS calibration curve was analyzed first, 10 to 1000ppb of the 2,6-DFBA were used to prepare the 2,6-DFBA TMS solutions, 2μl of 100ppm ethyl benzoate in ethyl acetate was added as internal standard. A wo microliter volume of the derivative solution was then injected onto the GC/MS column.

2.3.3.5 C-Well Sample Analysis

After the generation of the GC/MS calibration curve, the same method was used to prepare the 2,6-DFBA TMS solution from the C-Well sample. After the addition of the 2μl of 100ppm ethyl benzoate, two microliters of the derivative solution was analyzed, and the result was compared with the HPLC analysis.

2.3.3.6 HPLC Analysis of the Solvent Extraction Efficiency

2,6-DFBA is usually being put into the wells as tracer to follow the movement of the ground water, then the water sample is pumped out of the C-Well and analyzed by HPLC. In this study, water sample can't be directly injected onto the GC. In addition, the silylation of the 2,6-DFBA can not allow the presence of the water. So the first step of the pretreatment procedure is to extract the 2,6-DFBA from a water solution into an organic solvent for the silylation reaction to take place. During the liquid-liquid extraction, there may be some loss of the 2,6-DFBA, which can be calculated by evaluating the amount of the 2,6-DFBA left over in the aqueous phase after the extraction. HPLC was utilized for this purpose.

A Spectra-Physics HPLC with a UV detector was used to analyze the aqueous phase after the extraction. Two milliliter of the aqueous phase solutions, after extraction from the 2,6-DFBA standards, were injected onto the HPLC column. Table 2.8 and 2.9 contain the HPLC parameters.

Table 2.8 HPLC Parameters (I)

Column: LC-ABZ
Pump: SP8800
Pressure: 760 Psi
Flow rate: 1.0 ml/min
Run time: 10min
Injection volume: 50µl
UV/Vis detector: 230nm
Solvent: 72% KH_2PO_4 (0.01M) @pH 2.5
28% Methanol

Table 2.9 HPLC Parameters (II)

Column: LC-ABZ
Pump: P2000 quat gradient
Pressure: min.0 to max.6000psi
Delay volume: 0.00
Flow rate: 1.0 ml/min
Run time: 7 min
Injector: AS 1000 fixed loop
Injection mode: Full loop
Injection volume: 100µl
UV 100 UV/Vis detector: 230nm
Solvent profile: Linear
Solvent: 45% KH_2PO_4 (0.01M) @pH 2.5
55% Methanol

CHAPTER 3

RESULTS AND DISCUSSION

3.1 The Identification of 2,6-DFBA Silyl Derivative by GC/MS

The fragmentation pattern of 2,6-DFBA is shown below in Figure 3.1. In the EI mode of operation, the 2,6-DFBA vapor was bombarded with a beam of energetic electrons generated by the filament. By exchange of energy during the collision, an electron was removed from the molecule to form the molecular ion, then the further decomposition of the molecular ion occurred and fragment ions were formed.

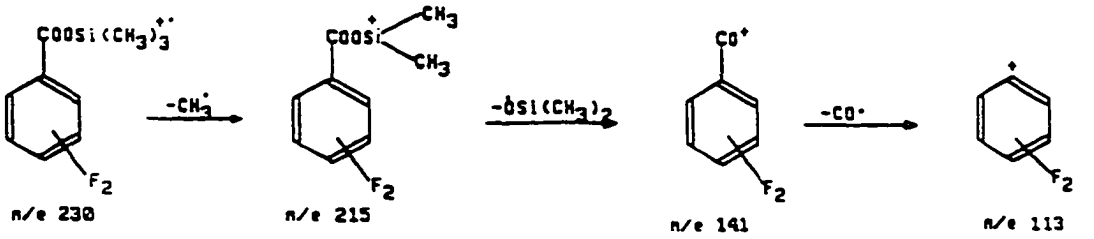


Figure 3.1 The fragmentation pattern of 2,6-DFBA TMS derivative.

Under the GC/MS conditions listed in Table 2.6, two microliter volumes of the 2,6-DFBA TMS solution, made from the 100ppb 2,6-DFBA standard, was analyzed. The 2,6-DFBA TMS peak yielded at 5.72 minutes, which is scan 226. The mass spectra

of 2,6-DFBA TMS is shown in Figure 3.2, the peak of 2,6-DFBA TMS derivative has the major peaks at m/e 141 and m/e 215, which are the $[M\text{-}OSi(CH_3)_3]^+$, and $[M\text{-}CH_3]^+$ respectively.

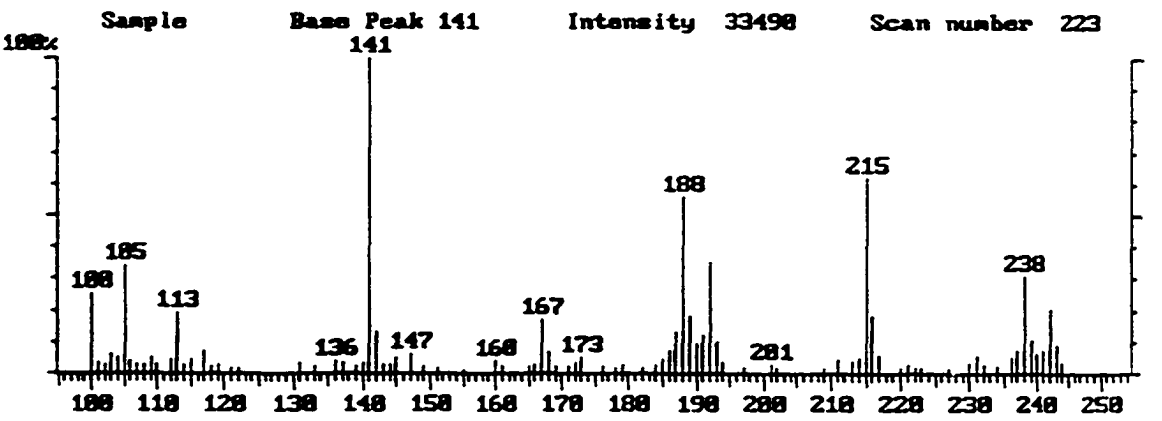


Figure 3.2 Mass spectra of 2,6-DFBA TMS derivative.

The ethyl benzoate was also identified since it was used as the internal standard. Five ppm of the internal standard ethyl benzoate prepared in ethyl acetate was injected onto the GC/MS column under the same condition. The peak yields at scan 192. The mass spectra of ethyl benzoate is shown in Figure 3.3. Ethyl benzoate has the major peak at m/e 105 and m/e 150.

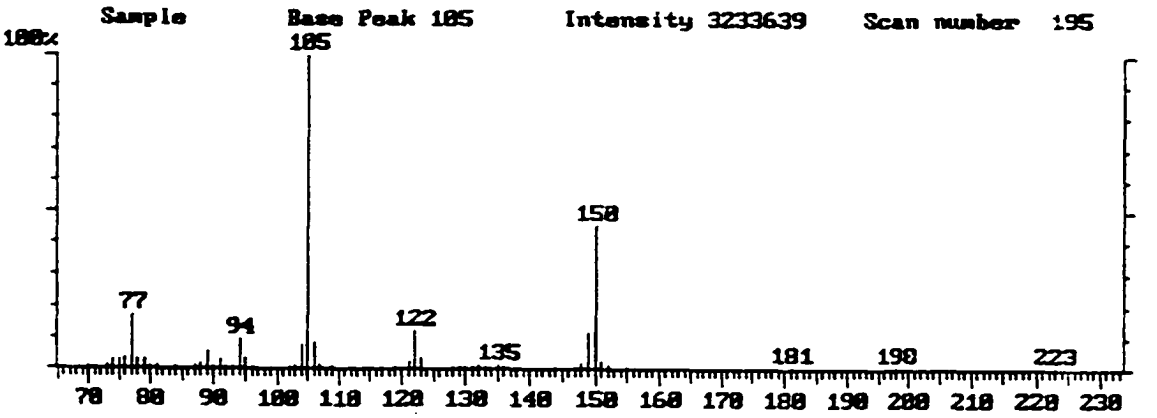


Figure 3.3 Mass spectra of ethyl benzoate.

3.2 Yield of 2,6-DFBA as a Function of Silylation Reaction Time

The experiment was repeated twice under the same conditions (Table 3.1). In the first experiment, 2,6-DFBA TMS derivative solutions were analyzed after 1 hour and 24 hours. The TMS solutions were made from 20ppb, 40ppb, 60ppb, 80ppb, 100ppb, and

Table 3.1 2,6-DFBA TMS reaction time test I, 1 hour reaction response compared with 24 hours reaction response.

CONC. OF 2,6-DFBA, PPB	2,6-DFBA TMS RESPONSE(R1), 1HR.	2,6-DFBA TMS RESPONSE (R2), 24HR.	[R2/R1-1]*100%
20	16,122	17,387	8
40	29,904	31,137	4
60	49,901	52,022	4
80	60,728	63,396	4
100	71,929	74,884	4
150	116,920	121,575	4

150ppb. The responses of 2,6-DFBA TMS after the 1 hour silylation reaction are compared with the responses after 24 hours in Figure 3.4. The results show that the differences between the two reaction times were between 4% to 8%.

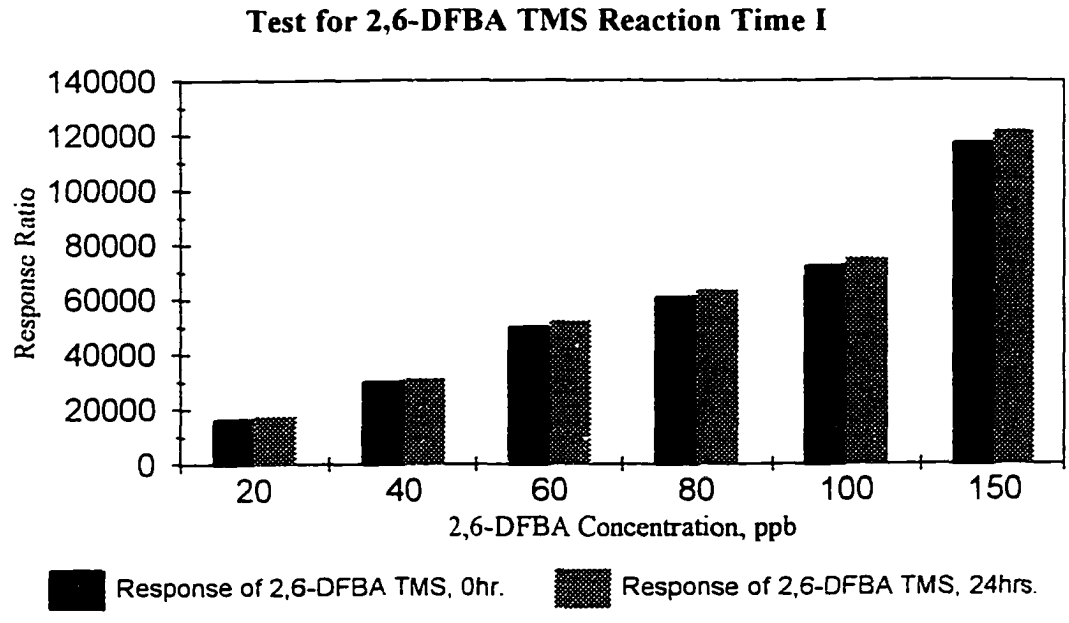


Figure 3.4 Silylation reaction test I of 2,6-DFBA TMS derivatives on GC/MS at 1 hr. and 24 hrs. reaction time.

The test was repeated and the 2,6-DFBA TMS solutions were from the 20ppb, 40ppb, 80ppb, and 200ppb of 2,6-DFBA standards. The result shows that the difference of 1 hour and 24 hours reaction are between 2% to 4% (Table 3.2 and Figure 3.5).

Table 3.2 2,6-DFBA TMS reaction time test II, 24 hours response compared with 1 hour response.

CONC. OF 2,6-DFBA, PPB	2,6-DFBA TMS RESPONSE (R1), 1HR.	2,6-DFBA TMS RESPONSE (R2), 24HR.	[R1/R2-1]*100%
20	13,264	12,990	2
40	25,731	24,670	4
80	51,883	50,854	2
200	165,308	164,647	0

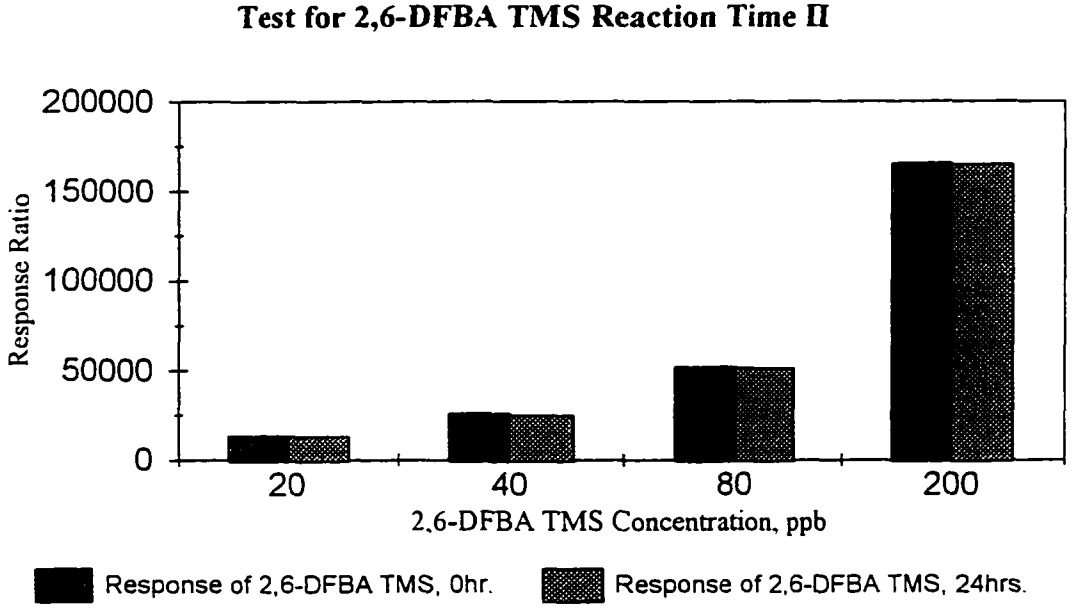


Figure 3.5 Silylation reaction test II of 2,6-DFBA TMS derivatives on GC/MS at 1 hr. and 24 hrs. reaction time.

Those tests proved that the changes of 2,6-DFBA TMS responses in 24 hours was within ±8% of the 1 hour responses, therefore 1 hour was used as the acceptable time to do the silylation reaction.

3.3 GC/MS Detection Limits and Precision

Detection limits are defined as the smallest amount that can be detected within a stated confidence limit. Two detection limits, method detection limit (MDL) and limit of quantitation (LOQ) were determined in this study.

Method detection limit (MDL) is calculated using the formula (Method 200.8-Determination of Trace Elements in Waters and Wastes by ICP/MS, 1994):

$$MDL = S * T_{(n-1,\ 1-\alpha = 0.98)}$$

MDLs are calculated using the one-sided t test for which t = 3.14 at n-1 where n = 7 at 98% confidence interval. Two microliter volumes of the 2,6-DFBA TMS, made from 5ppb 2,6-DFBA standard (5ppb was used because it was close to the estimated quantitation limit), was used to perform this test. Seven portions of this solution were analyzed on GC/MS. The experiment was repeated with a new prepared solution (from 5ppb of 2,6-DFBA standard) on a different day (Table 3.3). The t value for (7-1) degrees of freedom at 98% confidence is 3.14.

For test I, the detection limits are calculated as follows.

$$MDL = 0.07 * 3.14 = 0.2\ ppb$$

$$LOQ = 10/3 * MDL = 0.7\ ppb$$

The same calculations were performed for test II

$$MDL = 0.09 * 3.14 = 0.3\ ppb$$

$$LOQ = 10/3 * MDL = 1\ ppb$$

Table 3.3 The detection limits test of 2,6-DFBA TMS on GC/MS, based on 5ppb of 2,6-DFBA standard. 5μL of 100ppm ethyl benzoate was added to the 100μL TMS derivative solution as internal standard. Seven replicates of 2,6-DFBA TMS solution were analyzed.

INJECTION	2,6-DFBA TMS CONC. PPB, I	2,6-DFBA TMS CONC. PPB, II
1	4.89	5.02
2	5.04	4.84
3	4.99	5.12
4	5.06	5.01
5	4.94	4.99
6	5.01	5.07
7	5.08	4.96
Mean	5.00	5.00
STD	0.07	0.09
RSD	1%	2%
MDL, ppb	0.2	0.3
LOQ, ppb	0.7	1

By taking the average of these two tests, the method detection limit of GC/MS is 0.3 ppb, and the limit of quantitation is 0.9 ppb.

Precision for 2,6-DFBA TMS at low concentration (5ppb) is 2% (Table 3.3) relative standard deviation (n=7).

3.4 2,6-DFBA TMS Stability Test

The 2,6-DFBA TMS solutions, prepared fresh from 40ppb, 200ppb (both analyzed after 30 minutes extraction), and 100ppb (after 60 minutes extraction) of 2,6-DFBA standards, were stored in refrigerator at 4°C and analyzed in 24 hours. The response of 2,6-DFBA TMS after 1 hour reaction is assumed as initial concentration C_0,

the response at later times are compared with the C_0, and the C/C_0 ratios are listed in Table 3.4. Figure 3.6 shows the concentration variations over 24 hours.

The stability test shows that the 2,6-DFBA TMS is relatively stable during the 24 hours. It is proved that 2,6-DFBA TMS solutions can be analyzed within the 24 hours period. However, to avoid the absorption of the water moisture and the dryness of the sample, the vials should be kept in a refrigerator during the whole analysis period, with the cap tightly screwed up and wrapped with parafilm.

Table 3.4 Stability test for 2,6-DFBA TMS. 5μL of 100ppm ethyl benzoate was added to 100μL TMS solution. 40ppb and 200ppb standards were analyzed after 30 minutes extraction, and 100ppb was analyzed after 60 minutes extraction.

TIME HR.	2,6-DFBA TMS C/C_0 From 40ppb 2,6-DFBA	2,6-DFBA TMS C/C_0 From 100ppb 2,6-DFBA	2,6-DFBA TMS C/C_0 From 200ppb 2,6-DFBA
1	1	1	1
2	0.97	1.03	1.05
3	1.04	1.02	1.05
4	0.88	1.03	1.06
6	0.96	1.00	1.04
9	1.00	0.99	1.08
24	1.09	0.99	1.06

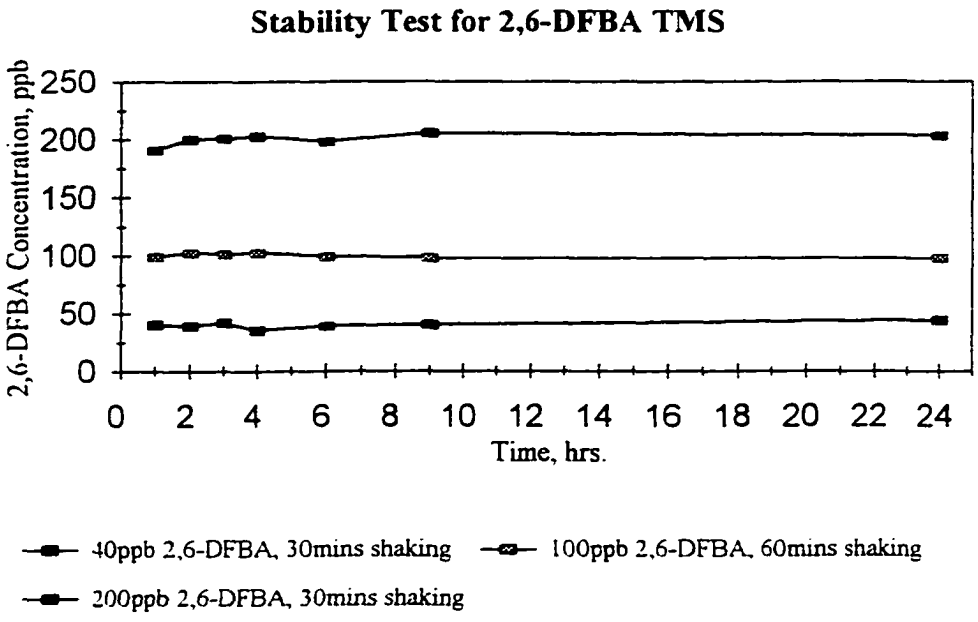


Figure 3.6 Stability test for the 2,6-DFBA TMS from 40ppb, 100ppb, and 200ppb of 2,6-DFBA standards.

3.5 Recoveries of 2,6-DFBA from Extraction

Since the sample pretreatment involves the liquid-liquid extraction, HPLC was used to analyze the aqueous phase to find the recovery of the 2,6-DFBA after this extraction.

First, the calibration curve of 2,6-DFBA is generated on the HPLC in the concentration ranged from 0 to 200ppb, calibration curve is shown in Figure 3.7. A linear relationship is achieved with the regression coefficient 0.9999.

Second, the aqueous phase of 2,6-DFBA standards after extraction was analyzed on HPLC. Twenty milliliters of each 2,6-DFBA standard solution (ranges from 0 to 200ppb) was acidified to pH 1.9 to 2.3, then dried ethyl acetate was added for

extraction. The left over aqueous layer solution was then injected onto the HPLC column. Chromatograms are shown in the Appendix Figure 12 through Figure 21.

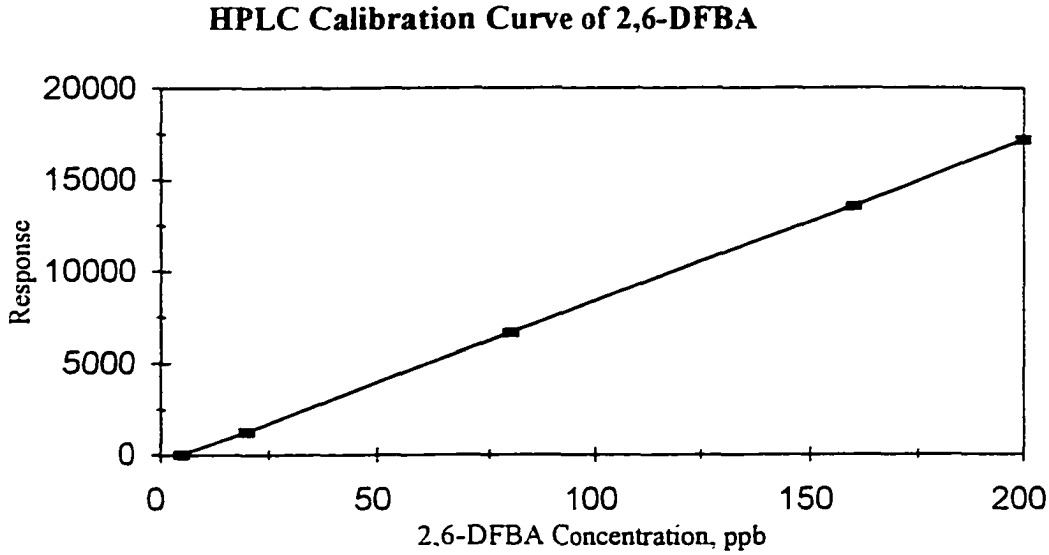


Figure 3.7 Calibration curve of 2,6-DFBA analysis on HPLC/UV.
Response = X Coef.*Conc + Const.
X Coef. = 87.90±0.50, Const. = -470, Corr. Coeff = 0.9999

The results show that over 65% of the 2,6-DFBA is being transferred into organic layer when using ethyl acetate and a contact time of 30 minutes (Table 3.5). Higher transfer efficiencies are observed for the high concentration of 2,6-DFBA solutions, and lower transfer efficiencies are observed for the less concentrated 2,6-DFBA solutions. Results are not available for 100ppt and 10ppb of 2,6-DFBA standards because they were below the instrument quantitation limit, which is about 10ppb.

Table 3.5 HPLC test for extraction efficiency of 2,6-DFBA from 20mL standard solution to ethyl acetate after 30 minutes extraction.

2,6-DFBA STD PPB	INJ.	RT MIN	EXTRACTS RESPONSE	EXTRACTS CONC. PPB	EXTRACTION EFFICIENCY %
10	1	5.000	0	0	NA
10	2	5.000	0	0	NA
50	1	5.057	434	16.8	66
50	2	5.027	297	15.3	69
100	1	4.981	975	22.7	77
100	2	5.021	1,049	23.6	76
1,000	1	5.002	14,405	170.5	83
1,000	2	4.998	14,853	175.5	83

The transfer efficiency can be improved by using a longer shaking time of 60 minutes to extract the 2,6-DFBA into the ethyl acetate layer. Table 3.6 shows that over 80% extraction efficiency can be achieved between the concentration range of 60ppb and 200ppb, with more concentrated solutions yielding higher efficiencies. Figure 3.8 shows the extraction efficiency increases with the increase in analyte concentration.

The 20ppb and 40ppb of the 2,6-DFBA standards only gave 58% and 70% extraction efficiency, the reason may be because of the uncertainty associated with the number of those area counts. Since in calibration curve, the area count of 20ppb 2,6-DFBA peak was 1,230, so the area count of 5ppb 2,6-DFBA is supposed to be about 300, however, the actual response of 5ppb was zero. Therefore, the area counts data from 179 to 602, which were the responses of 20ppb and 40ppb of 2,6-DFBA extracts, are probably unreliable, and the extraction efficiency calculations are suspect. The calculated extraction efficiencies for 100ppb, 150ppb and 200ppb are more realistic.

Table 3.6 HPLC test for extraction efficiency of 2,6-DFBA from 20mL 2,6-DFBA standard solution to ethyl acetate after 60minutes extraction.

2,6-DFBA STD PPB	INJ.	RT MIN	EXTRACTS RESPONSE	EXTRACTS CONC. PPB	EXTRACTION EFFICIENCY %
20	1	4.362	179	7.4	63
20	2	4.385	315	8.9	55
60	1	4.398	486	10.9	82
60	2	4.390	602	12.2	80
100	1	4.399	757	14.0	86
100	2	4.387	598	12.1	88
150	1	4.397	1,107	17.9	88
150	2	4.376	1,133	18.2	88
200	1	4.398	1,547	22.9	89
200	2	4.395	1,558	23.1	89

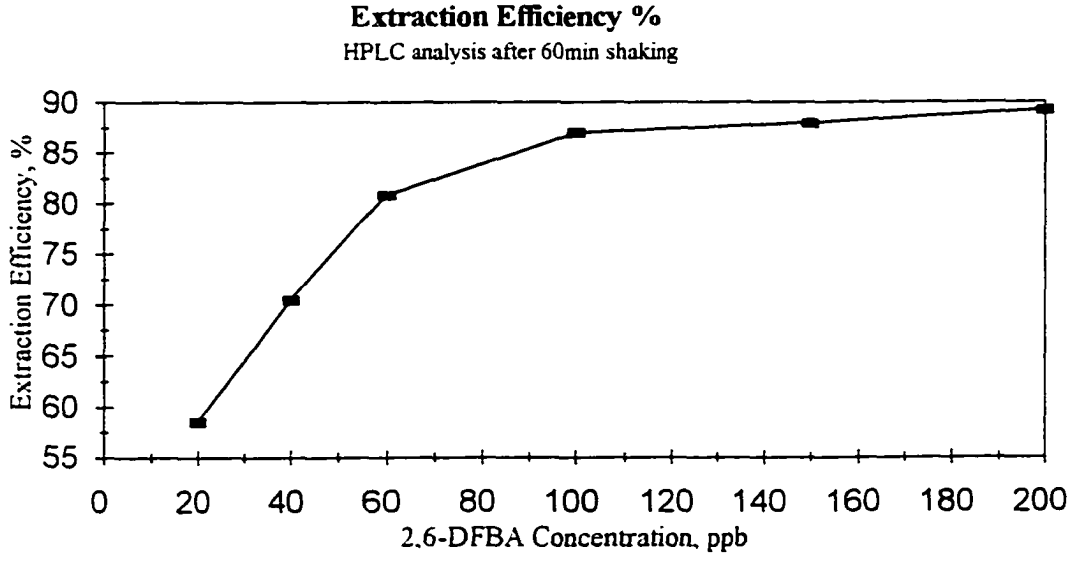


Figure 3.8 Extraction efficiency of 2,6-DFBA into ethyl acetate after 60 minutes liquid-liquid extraction.

When ethyl acetate is used for extraction, it will absorb some water, and after evaporation, a small amount of water might be present in the dried sample. The presence of water will kill the TMS reaction and reverse the direction of the reaction. However, Weiss (Weiss and Tambawala, 1972) showed that this problem can be solved by adding large excess of BSTFA into the reaction.

3.6 GC/MS Instrument Calibration

Standardization of instruments is essential in obtaining accurate analyses. Two of the most commonly used calibration techniques are external calibration and the internal standard method.

In the external calibration curve technique, a series of standard solutions containing known concentrations of the analytes are prepared. These solutions should cover the concentration range of interest and have a matrix composition as similar to that of the sample solutions as possible. A blank solution containing only the solvent matrix is also analyzed, and the net readings–standard solutions minus blank–versus the concentrations of the standard solutions are plotted to obtain the calibration curve.

The internal standard is used to minimize differences in the physical properties of a serious of sample solution that contain the same analyte. In this method, a fixed quantity of a pure substance is added to the samples and standard solutions alike. The responses of the analyte and internal standard, each corrected for background, are determined, and the ratio of the two responses is calculated. A plot of the response ratio

as a function of the analyte concentration yields the calibration curve. The calibration curve is prepared every week due to changes in instrument response.

The internal standard is added at the beginning of an analysis to allow for dissolution, mixing, and any other reactions to occur before a measurement is made. 100ppm of ethyl benzoate was used as the internal standard in this study, because it is a substance similar to the analyte 2,6-DFBA TMS, with an easily measurable signal that did not interfere with the response of the 2,6-DFBA TMS. An internal standard hopefully responds in a manner similar to the analyte and corrects for any variables that may affect the detector response. Ethyl benzoate was added to give a concentration which is the same order of magnitude as that of the 2,6-DFBA TMS in order to minimize error in calculating the response ratios.

The calibration analysis were performed using the 2,6-DFBA TMS with different extraction periods, 30 minutes, and 60 minutes the silylation procedure and the GC/MS conditions are listed in section 2.3.1 and 2.3.3. A linear relationship is achieved between the response ratio and concentration of the 2,6-DFBA TMS.

The calibration curve for the 2,6-DFBA TMS derivative after 30 minutes of shaking is shown in Figure 3.9 (the concentration is based on the 0-200ppb of 2,6-DFBA standards). A linear relationship is achieved with the correlation coefficient of 0.9984.

The calibration curve for 2,6-DFBA TMS derivative after 60 minutes of shaking is shown in Figure 3.10, the concentration is based on the 0-150ppb of 2,6-DFBA standards. A linear relationship is achieved with the correlation coefficient of 0.9995.

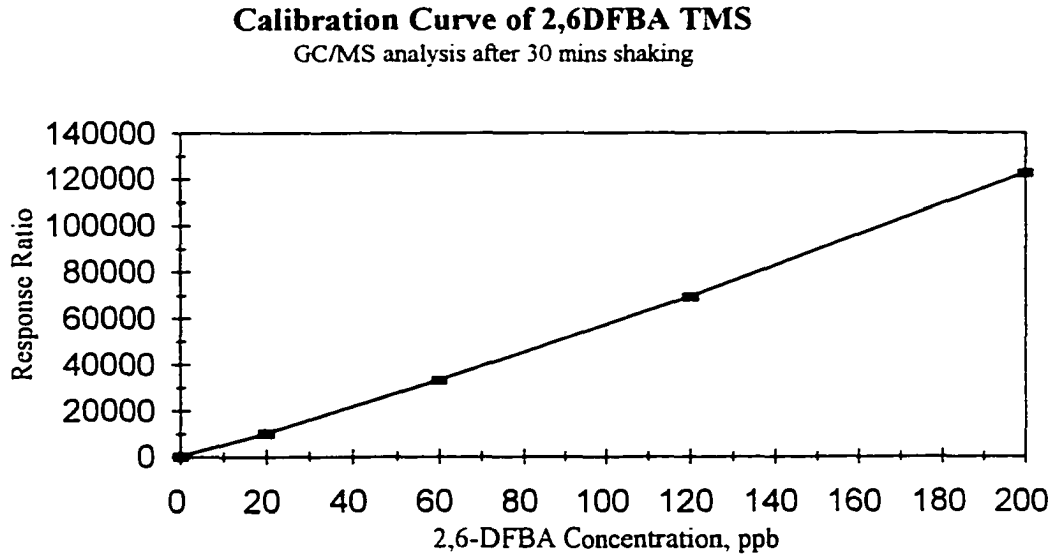


Figure 3.9 Calibration curve of 2,6-DFBA TMS analysis on GC/MS, after 30 mins extraction.
Response = X Coef.*Conc. + Const.
X Coef. = 614.22±14.16, Const. = -2149, Corr. Coeff = 0.9984

The chromatogram of the calibration standards are shown in Appendix Figure 22 through Figure 27.

GC/MS calibrations are based on the assumption that the yields and recoveries of 2,6-DFBA TMS were 100% quantitative. However, there are two major steps that need to be considered. First, some of the 2,6-DFBA may have been lost during the extraction process. Second, the silylation reaction probably was not 100 percent complete. Therefore, a more realistic estimation of the 2,6-DFBA TMS was calculated by the following equation.

$$CONC_{2,6\text{-}DFBA\ TMS} = CONC_{2,6\text{-}DFBA} \times Extraction\ efficiency \times Silylation\ reaction\ efficiency$$

The extraction efficiency was explained in the previous section, but, the silylation reaction efficiency is difficult to quantify because there is no 2,6-DFBA TMS derivative available commercially.

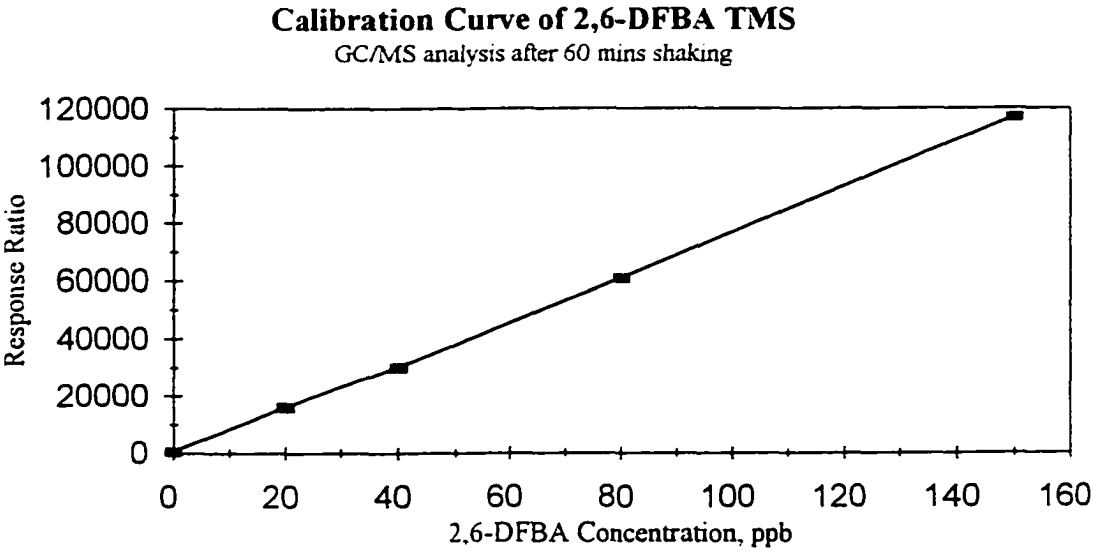


Figure 3.10 Calibration curve of 2,6-DFBA TMS analysis on GC/MS, after 60 mins. extraction.
Response = X Coef.*Conc + Const.
X Coef. = 774.72±9.58, Const. = -102, Corr. Coeff = 0.9995

3.7 C-Well Sample Analysis

The C-Well sample dated on Mar. 3, 1997 was analyzed on both GC/MS and HPLC for comparison.

20 mL of C-Well water sample was prepared using the same procedure stated in section 2.3.1. and 2μL of the 2,6-DFBA TMS derivative solution was analyzed on GC/MS under the conditions in Table 2.6. GC/MS analysis of the C-Well sample concentration showed that there was 93ppb of 2,6-DFBA present (Calibration Curve see

Figure 3.8). Table 3.7 shows the result of replicate GC/MS analyses. HPLC analysis of the same sample yielded 70±3 ppb. There is 33% difference between the two results.

The reason to cause this difference might be because of the variation of the two instruments. The 2,6-DFBA was analyzed by HPLC in March, 1997 and analyzed on GC/MS in October, 1997, the solution might became more concentrated after six months storage in glass bottle in refrigerator. In addition, HPLC and GC/MS analysis were using different standard solutions, which were from different sources and made by different people.

Table 3.7 GC/MS Analysis of C-Well sample.

RUN	IS RESPONSE	2,6-DFBA TMS RESPONSE	NORMALIZED RESPONSE	CONC. OF 2,6-DFBA, PPB
1	58,096	71,289	71,432	92
2	57,439	71,120	72,078	93

CHAPTER 4

CONCLUSIONS AND FUTURE WORK

The silylation method combined with GC/MS can be used for determination of 2,6-DFBA in groundwater. The method offers two major advantages over the HPLC method as follow.

1. Lower method detection limits, 0.2μg/L and lower limit of quantitation, 1μg/L were achieved (these detection limits are based on the about 50% extraction efficiency of 5 ppb 2,6-DFBA standard), which was compared with HPLC detection limit 3μg/L. This can reduce by one order of magnitude the concentration of 2,6-DFBA put into the ground water when conducting the ground-water tracer analysis.
2. GC/MS provides a positive identification of the 2,6-DFBA and could provide qualitative and quantitative information in the presence of chromatographic interferences in heavily contaminated ground waters.

However, this method has several drawbacks compared with the HPLC method.

1. At least two and half hours are needed for preparing each sample before the GC/MS analysis. In the HPLC method, the water sample is injected directly after filtration.
2. Even though the retention time for 2,6-DFBA is about 5~6 minutes, the sample analysis time needs 9 minutes for everything going through the column to prevent the carry over to the next injection, and the instrument needs 2~3 minutes to cool down to the initial temperature before next injection can be performed. HPLC sample analysis time is 6~7 minutes with the 2,6-DFBA peak coming out at 5~6 minutes. No extra time is needed in between each run.

By comparing the GC/MS method with HPLC, in case of large quantity or routine sample analysis, HPLC is the better choice for speed and ease of analysis. GC/MS offers the accurate fingerprinting analysis with a small quantity of sample analysis.

Future work should address 1) improving the extraction efficiency and 2) decreasing the sample preparation time by the same time assets the silylation reaction efficiency. The silylation reaction condition was performed at room temperature for 1 hour in this study, which could be modified by using higher temperature to increase the speed of sample reaction, but care needs to be taken to prevent the loss of analyte during the heating process. A internal standard added at the very beginning of the sample pretreatment could be used to evaluate the silylation reaction efficiency, and this compound should have the correspondent TMS derivative in the market.

Flash chromatography could be tested for improvement of the extraction efficiency. However, a suitable column packing material with proper affinity for the compound needs to be chosen for 2,6-DFBA to be easily absorbed on the column and to be able to be desorbed by the organic solvent ethyl acetate.

Solid phase extraction (SPE) using C-18 bonded phases as packing material might be another choice. The advantages of SPE over conventional liquid-liquid extraction include quicker sample processing, generally high specificity of sorbent-analyte interactions resulting a good prepurification of complex samples, economic use of solvent, and procedural simplicity, potentially reducing the risk of manipulation errors in routine assays (Gessner and Schmitt, 1995). However, the efficient recovery of extracted analytes from the SPE is significantly affected by factors such as carbon loading, silanophilic interactions, and quantity and type of SPE phase (Martin et al, 1997).

APPENDIX

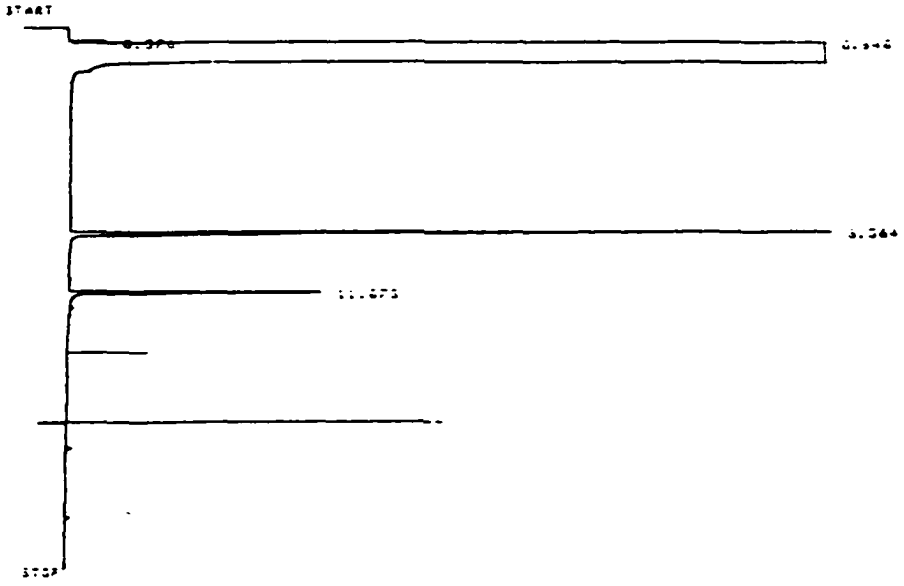


Figure 1. Chromatogram of GC/FID analysis of 2,6-DFBA and 2,6-DFBA methyl ester after 1 hr. methylating reaction.

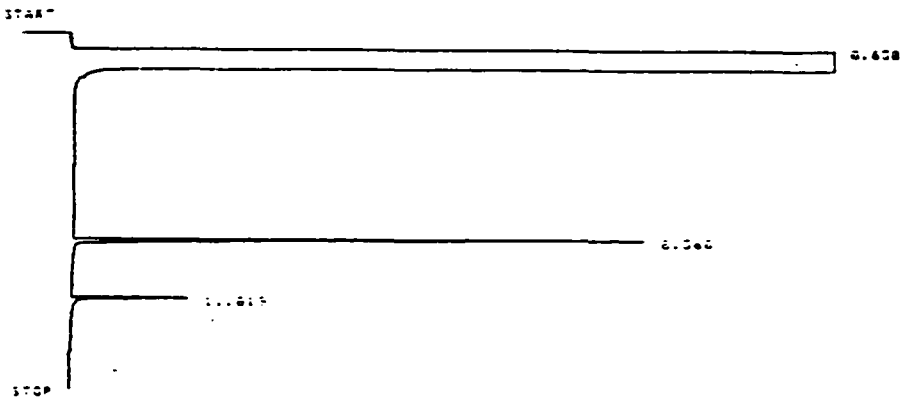


Figure 2. Chromatogram of GC/FID analysis of 2,6-DFBA and 2,6-DFBA methyl ester after 4 hr. methylating reaction.

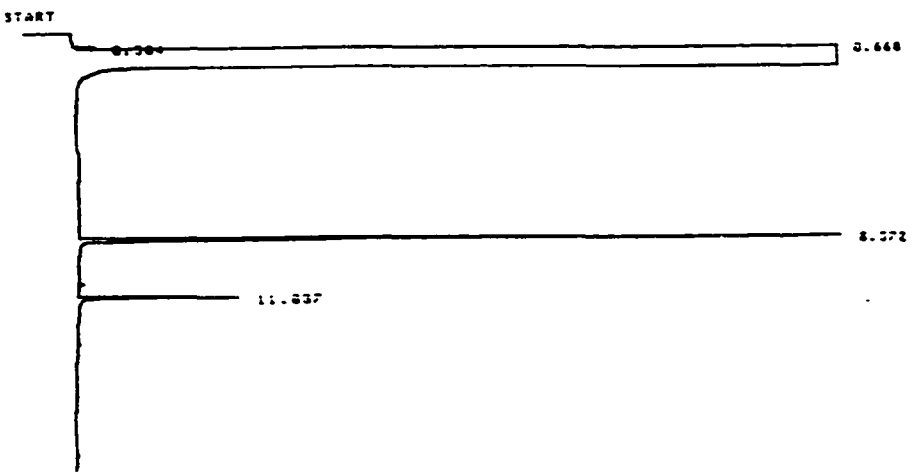


Figure 3. Chromatogram of GC/FID analysis of 2,6-DFBA and 2,6-DFBA methyl ester after 9hr. methylating reaction.

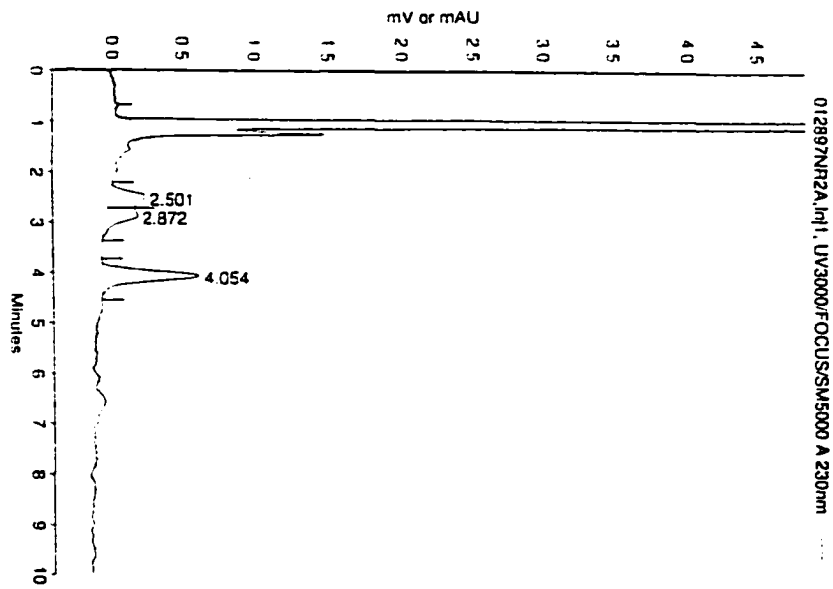


Figure 4. Chromatogram of HPLC analysis of 200ppb 2,6-DFBA standards for BF_3 methylation.

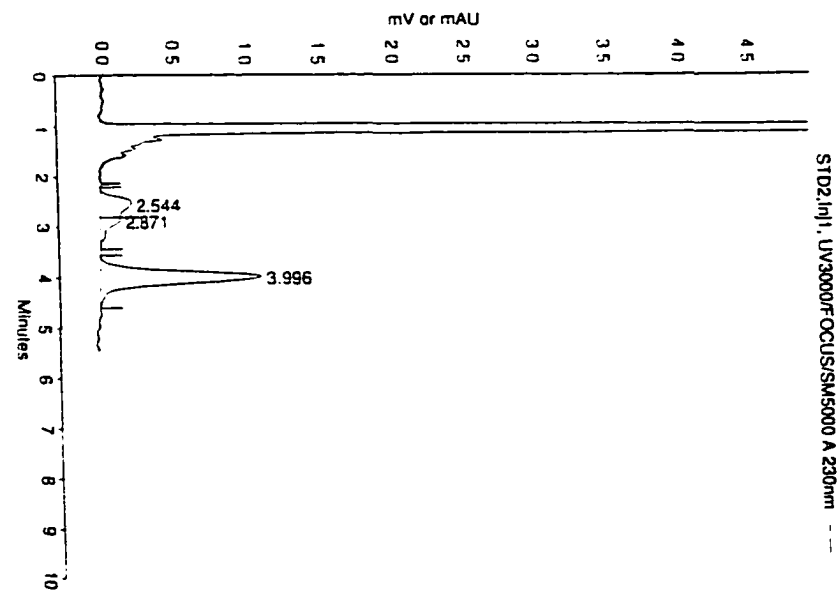


Figure 5. Chromatogram of HPLC analysis of 400ppb 2,6-DFBA standards for BF_3 methylation.

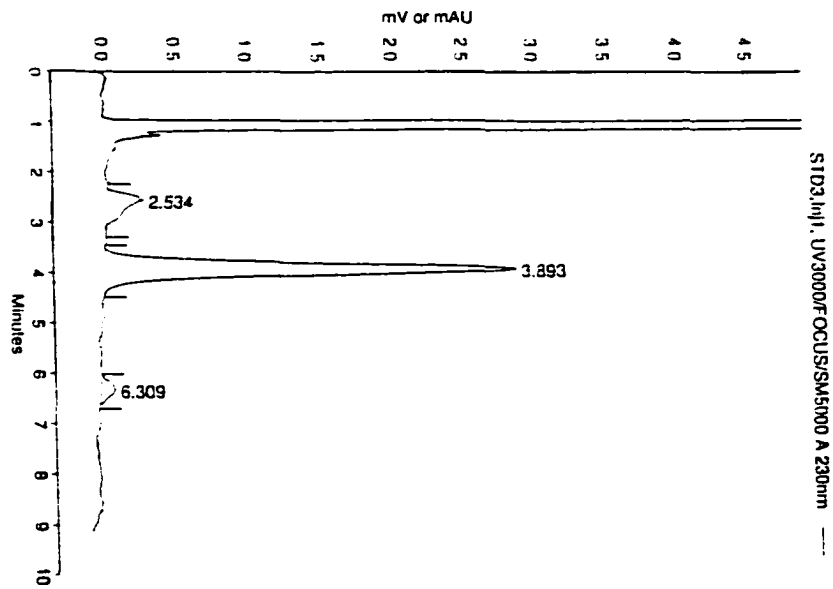


Figure 6. Chromatogram of HPLC analysis of 1000ppb 2,6-DFBA standards for BF_3 methylation.

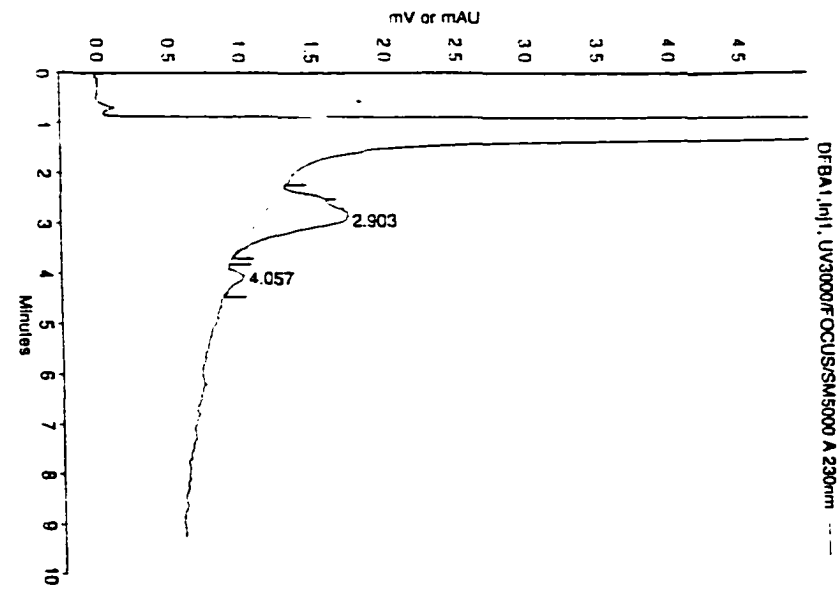


Figure 7. Chromatogram of HPLC analysis of non-reacted 1000ppb 2,6-DFBA in BF_3 metylation reaction after 1 minute.

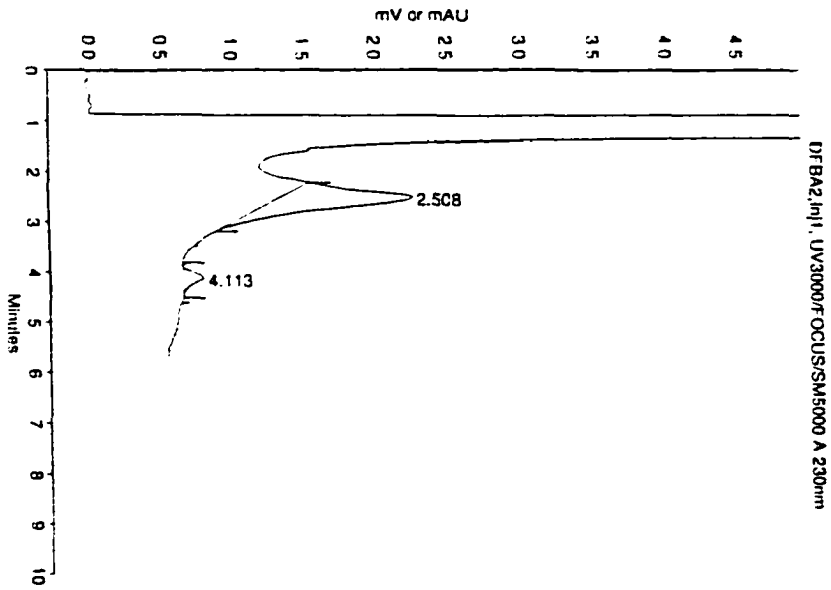


Figure 8. Chromatogram of HPLC analysis of non-reacted 1000ppb 2,6-DFBA in BF_3 metylation reaction after 2 minutes.

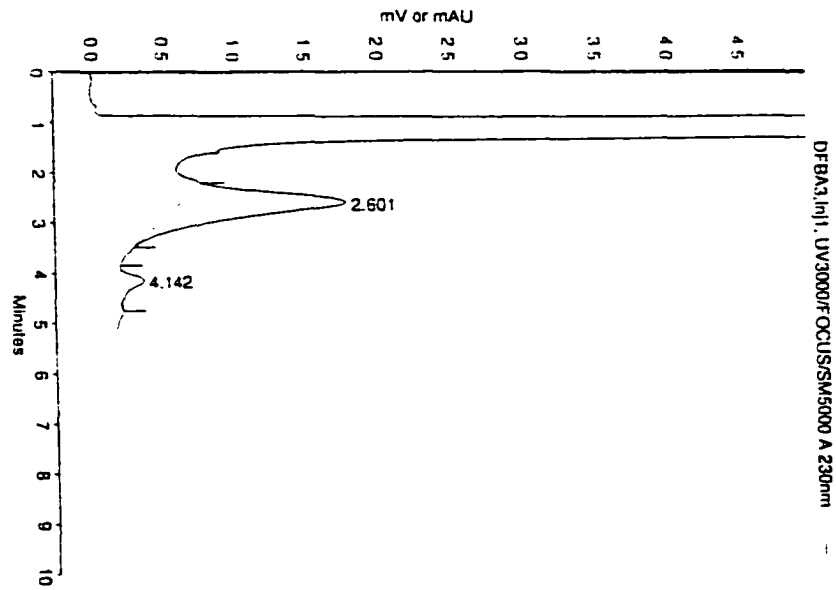


Figure 9 Chromatogram of HPLC analysis of non-reacted 1000ppb 2,6-DFBA in BF_3 metylation reaction after 3 minutes.

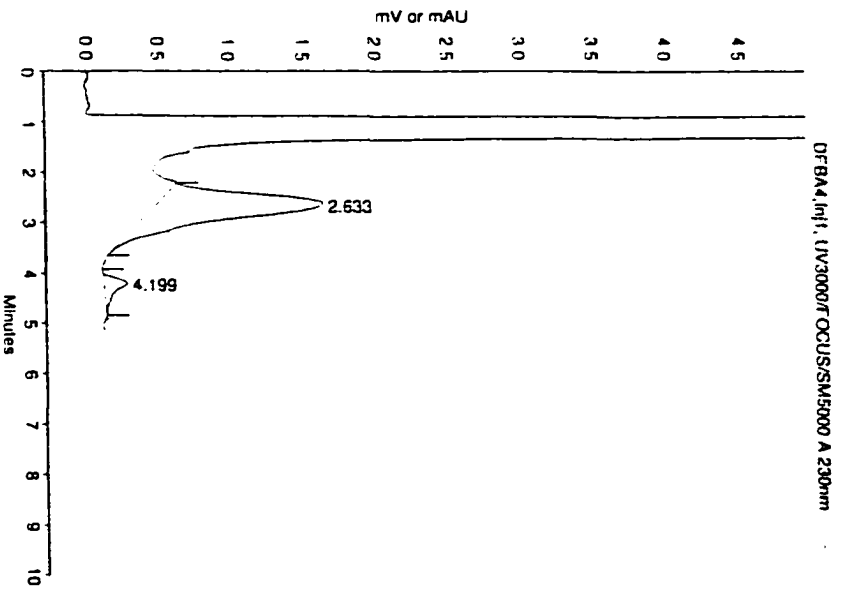


Figure 10. Chromatogram of HPLC analysis of non-reacted 1000ppb 2,6-DFBA in BF_3 metylation reaction after 4 minutes.

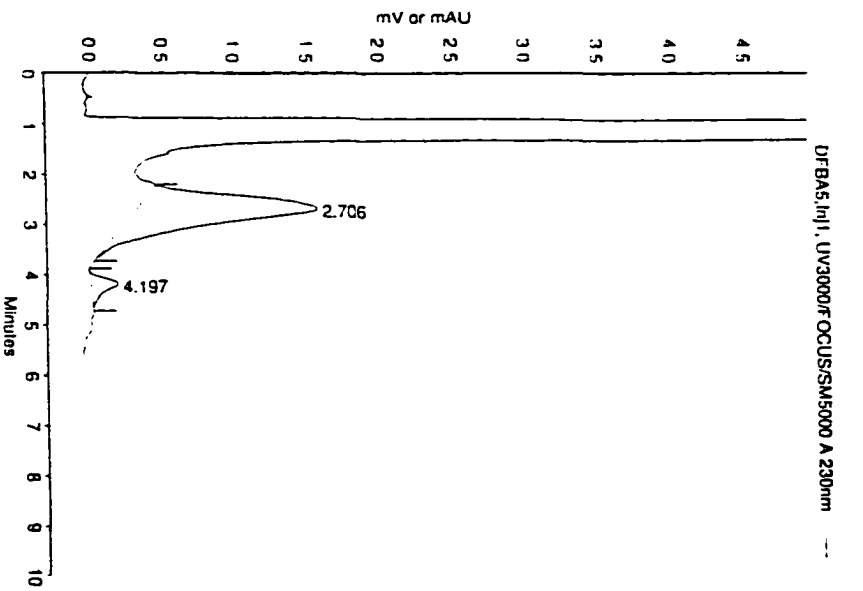


Figure 11 Chromatogram of HPLC analysis of non-reacted 1000ppb 2,6-DFBA in BF_3 metylation reaction after 5 minutes.

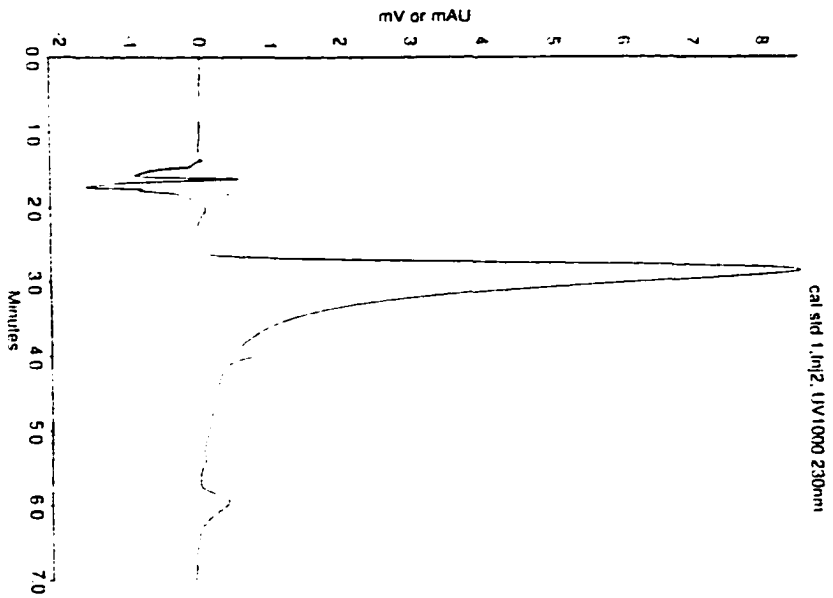


Figure 12 Chromatogram of HPLC calibration analysis of 5ppb 2,6-DFBA standard for silylation method.

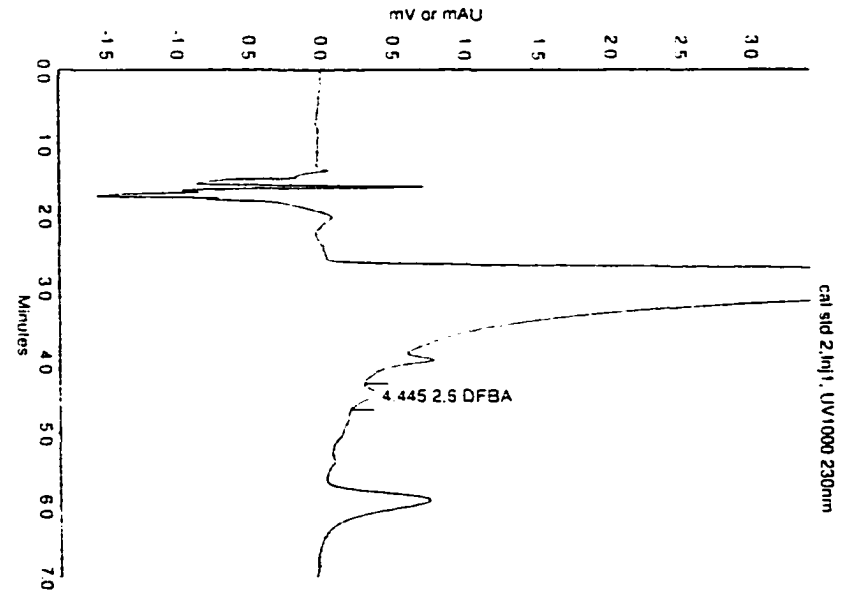


Figure 13 Chromatogram of HPLC calibration analysis of 20ppb 2,6-DFBA standard for silylation method.

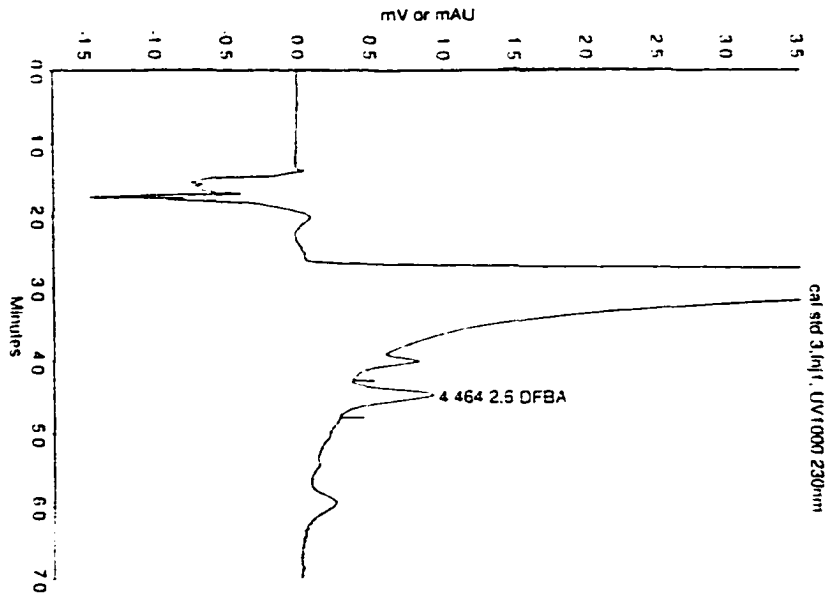


Figure 14 Chromatogram of HPLC calibration analysis of 80ppb 2,6-DFBA standard for silylation method.

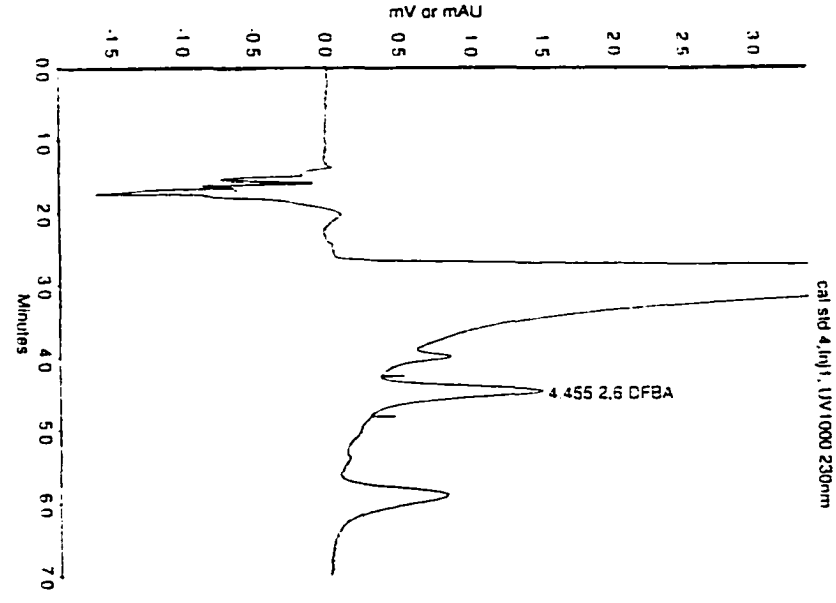


Figure 15 Chromatogram of HPLC calibration analysis of 160ppb 2,6-DFBA standard for silylation method.

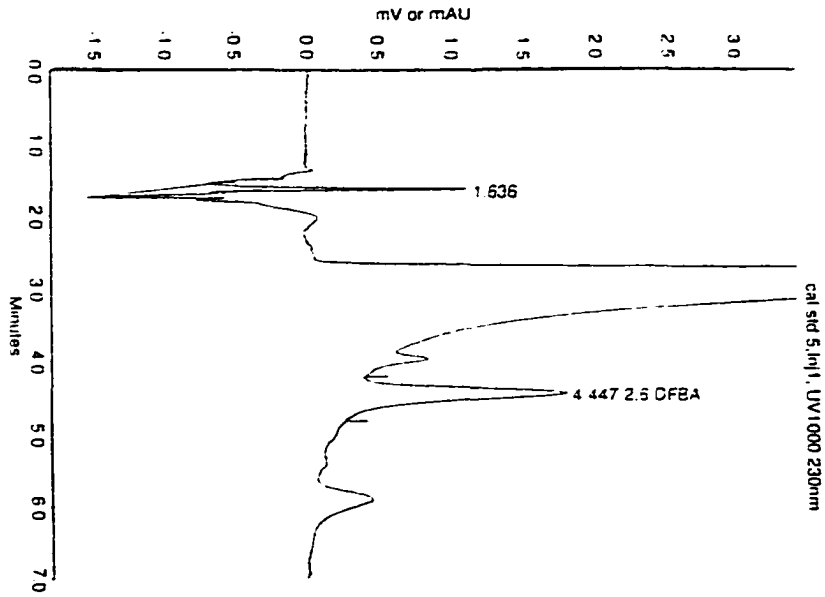


Figure 16 Chromatogram of HPLC calibration analysis of 200ppb 2,6-DFBA standard for silylation method.

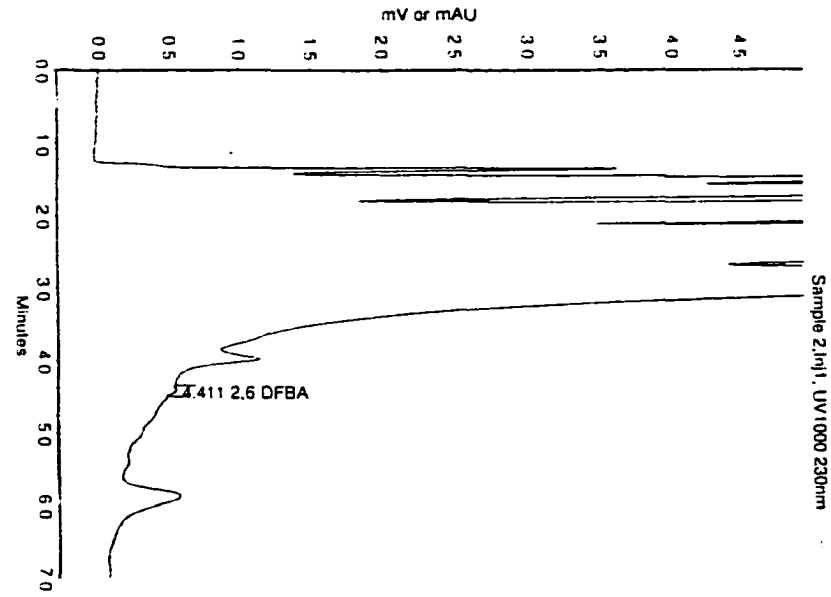


Figure 17 Chromatogram of HPLC analysis of 20ppb 2,6-DFBA extract after 60 minutes extraction in silylation method.

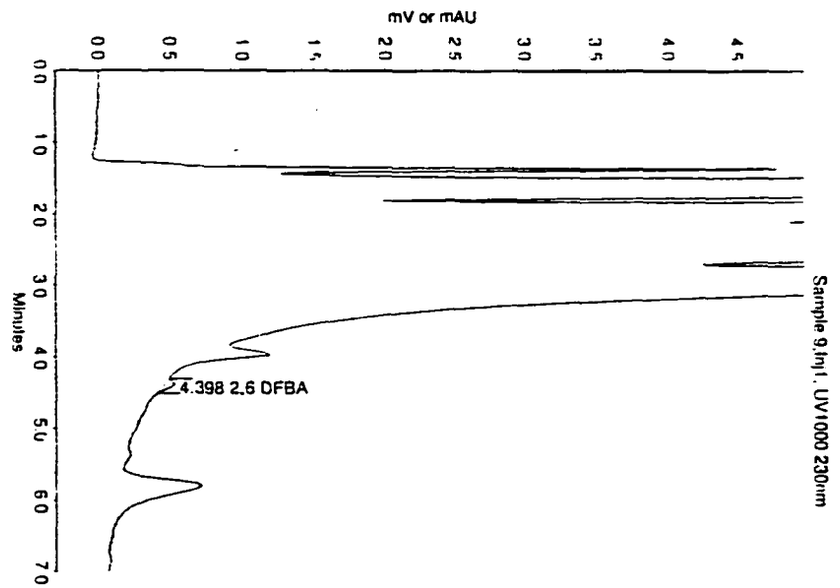


Figure 18 Chromatogram of HPLC analysis of 60ppb 2,6-DFBA extract after 60 minutes extraction in silylation method.

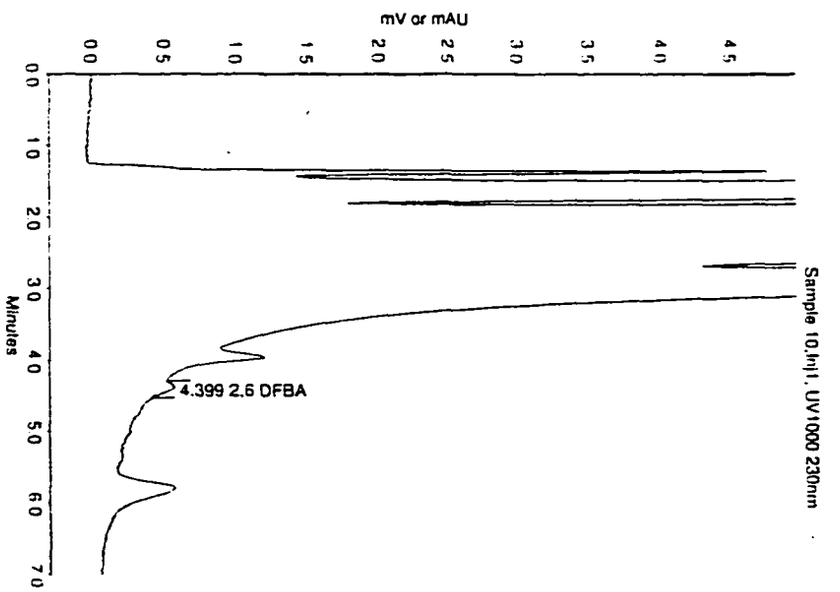


Figure 19 Chromatogram of HPLC analysis of 100ppb 2,6-DFBA extract after 60 minutes extraction in silylation method.

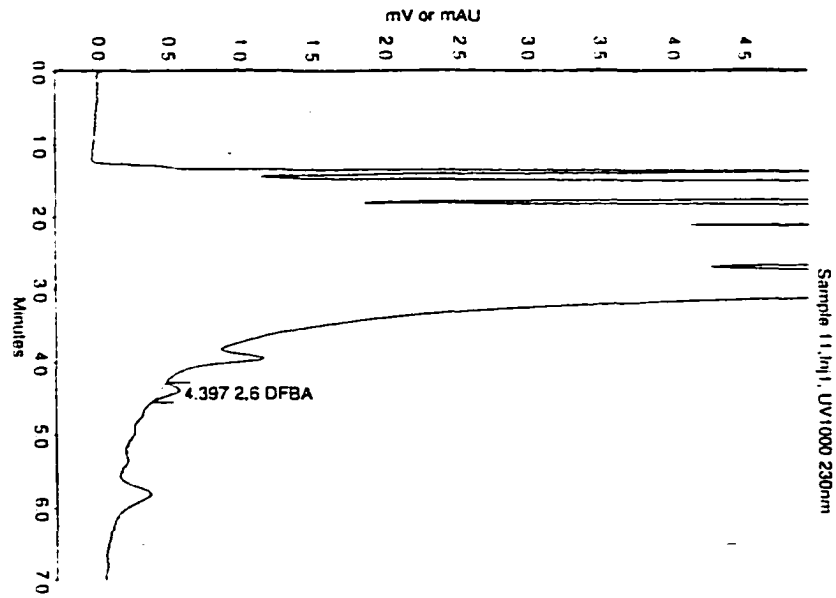


Figure 20 Chromatogram of HPLC analysis of 150ppb 2,6-DFBA extract after 60 minutes extraction in silylation method.

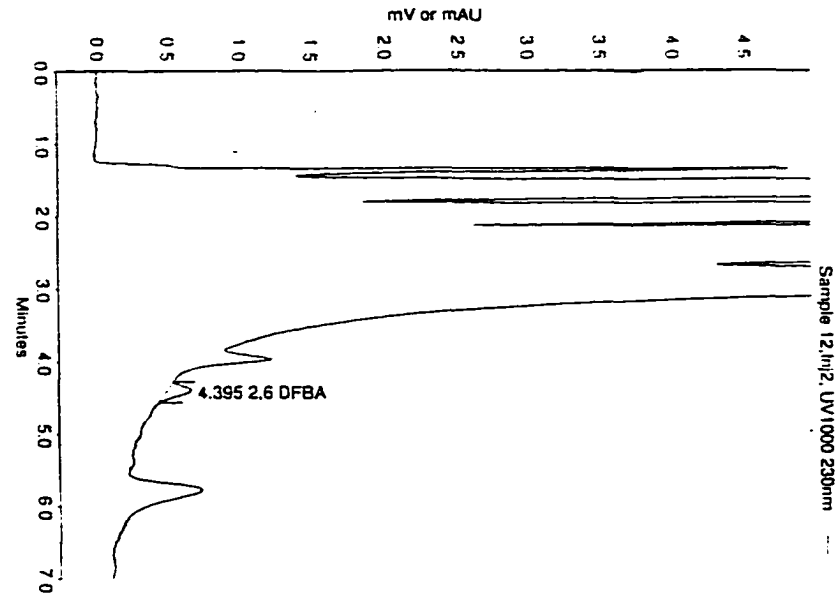


Figure 21 Chromatogram of HPLC analysis of 200ppb 2,6-DFBA extract after 60 minutes extraction in silylation method.

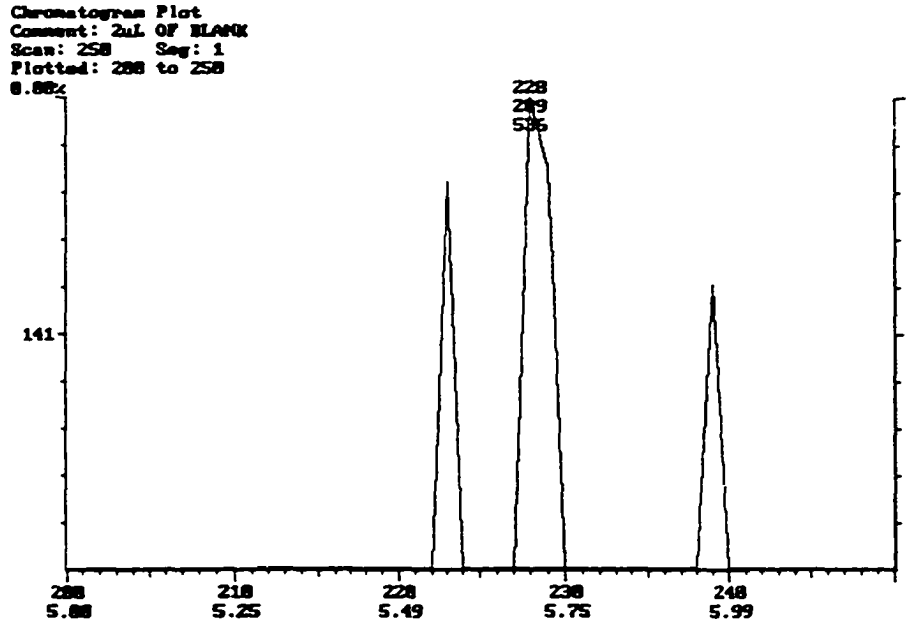


Figure 22 Chromatogram of GC/MS calibration analysis of reagent blank in silylation method.

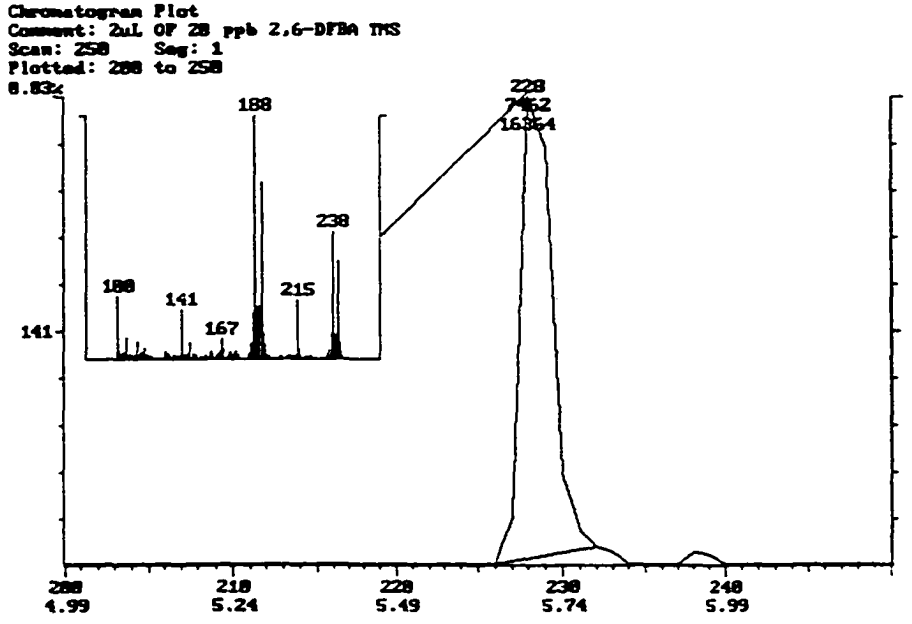


Figure 23 Chromatogram of GC/MS calibration analysis of 2,6-DFBA TMS, made from 20ppb 2,6-DFBA.

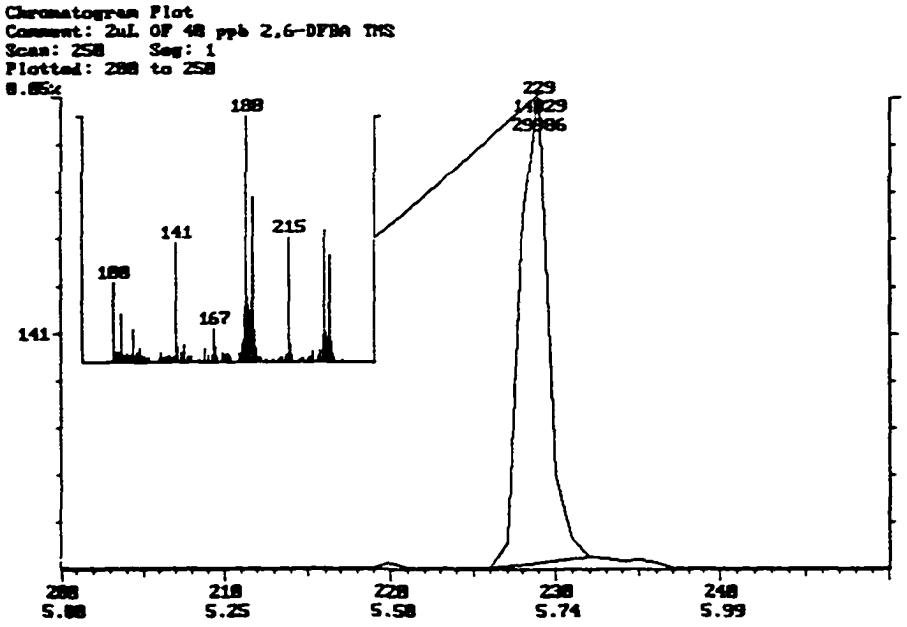


Figure 24. Chromatogram of GC/MS calibration analysis of 2,6-DFBA TMS, made from 40ppb 2,6-DFBA.

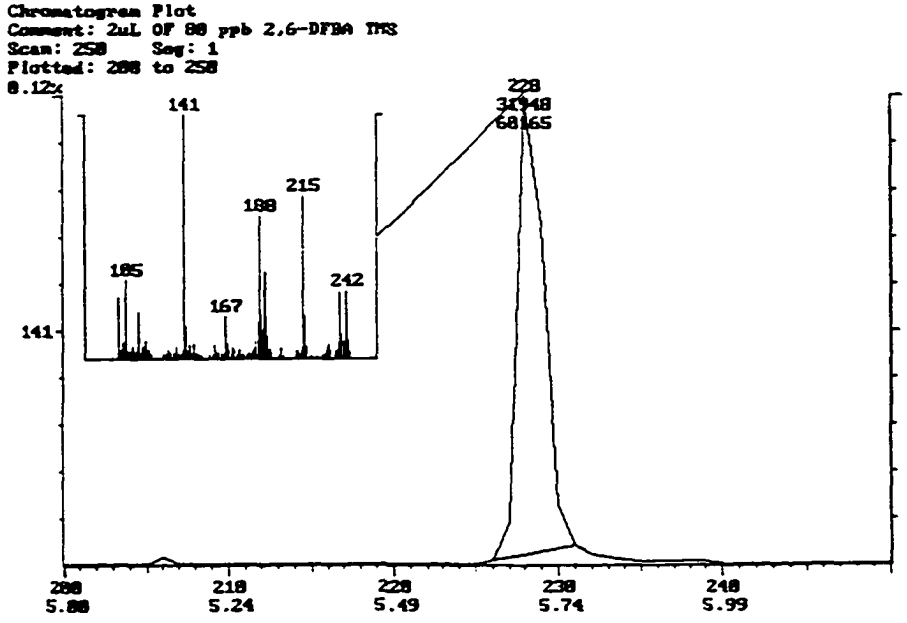


Figure 25 Chromatogram of GC/MS calibration analysis of 2,6-DFBA TMS, made from 80ppb 2,6-DFBA.

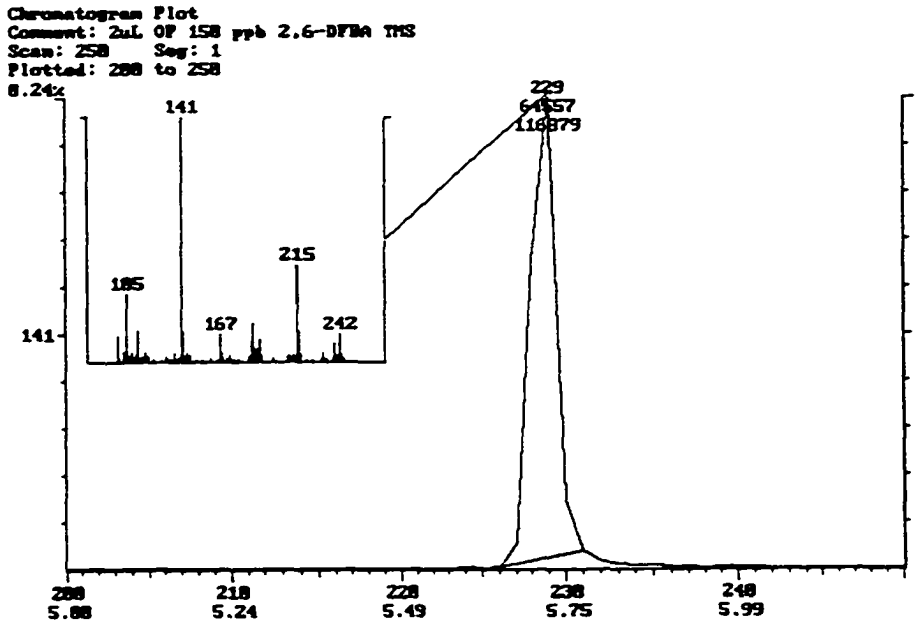


Figure 26 Chromatogram of GC/MS calibration analysis of 2,6-DFBA TMS, made from 150ppb 2,6-DFBA.

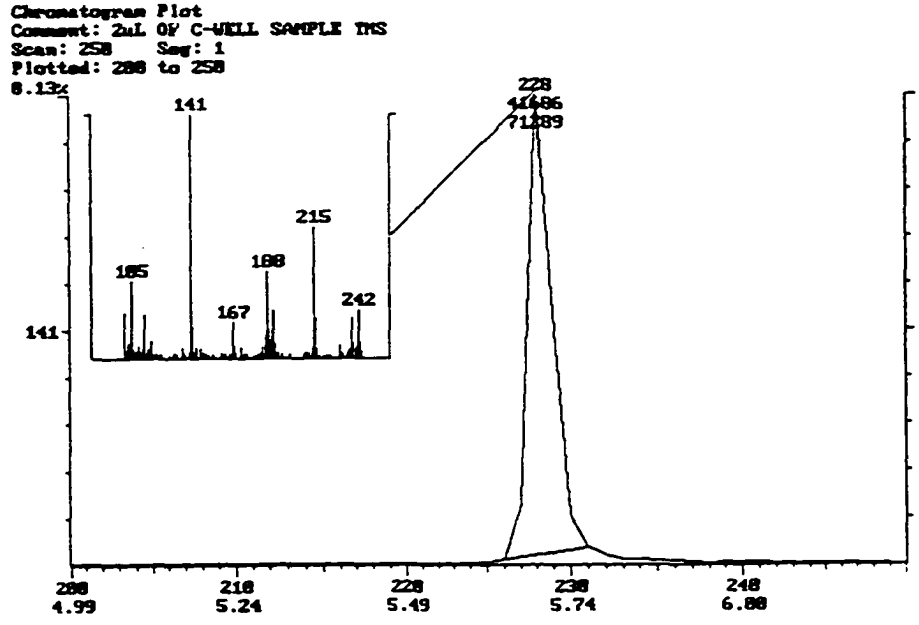
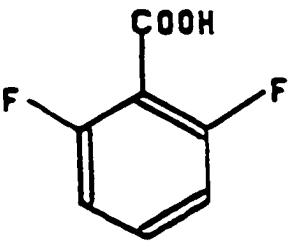
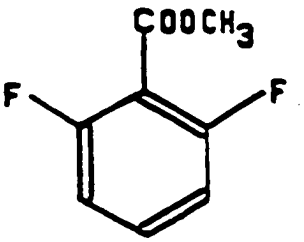


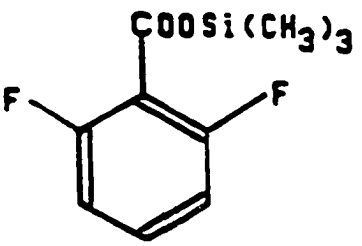
Figure 27 Chromatogram of GC/MS calibration analysis of 2,6-DFBA TMS, made from C-Well sample.



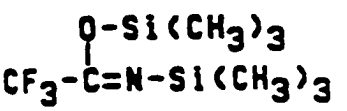
1) 2,6-DFBA



2) 2,6-DFBA methyl ester



3) 2,6-DFBA TMS derivative



4) BSTFA

Figure 28. The structure of 2,6-DFBA, 2,6-DFBA methyl ester, 2,6-DFBA silyl derivative, and BSTFA.

BIBLIOGRAPHY

Biemann, K. *Mass Spectrometry: Organic Chemistry Applications*. McGraw-Hill Book Company, New York. 1962.

Bowman, R. S. *Evaluation of Some New Tracers for Soil Water Studies*. Soil Sci. Soc. Am. J. Vol. 48. 1984a. pp. 987-993

Bowman, R. S. *Analysis of Soil Extracts for Inorganic and Organic Tracer anion via High Performance Liquid Chromatography*. J. Chromatography. 285. 1984b. pp. 467-477

Bowman, R. S. and J. F. Gibbens. *Difluorobenzoates as Nonreactive Tracers in Soil and Ground Water*. Ground Water. V.30. 1992. pp. 8-14.

Bowman, R. S. and R. C. Rice. *Accelerated Herbicide Leaching Resulting from Preferential Flow Phenomena and Its Implications for Ground Water Contamination*. Natl. Water Well Assoc., Dublin, OH. 1986. pp. 413-425.

Bremner, J. M. *Methods of Soil Analysis*. Part 2. Amer. Soc. of Agronomy, Madison, 1965. pp.1192-1197.

Cass, A.E.G., D. W. Ribbons, J. T. Rossiter, and S. R. William. *Biotransformation of Aromatic compounds. Monitoring Fluorinated Analogs by NMR. FEBS Letters.* Vol. 220. 1987. pp.353-357.

Cortes, H. J. *High Performance Liquid Chromatography of inorganic and organic anions using ultraviolet detection and an amino column.* J. Chromatogrphy 234. 1982. pp. 517.

Davis, Stanley N., Thompson, Glenn M., Bentley, Harold W., and Stiles, Gary. *Ground-Water Tracers — A Short Review.* Ground Water. Vol. 18. 1980. pp. 14-23.

Furniss, Brian S. VOGEL's Textbook Practical Organic Chemistry. 1989

Gessner, Mark O. and Anja L. Schmitt. *Use of Solid-Phase Extraction To Determine Ergosterol Concentrations in Plant Tissue Colonized by Fungi.* Applied and Environmental microbiology. Vol. 62. No. 2. Feb, 1996. pp. 415-420.

Ghauri, F. Y., C. A. Blackledge, R. C. Glen, I. D. Wilson, and K. Nicholson. *Quantitative Structure-Metabolism Relationship for Substituted benzoic Acids in rat. Computation Chemistry, NMR Spectroscopy and Pattern Recognition Studies.* Biochem. Pharmacol. Vol. 44. 1992. pp. 1935-1946.

Jandera, P. and J. Churacek. Gradient Elution in Column Liquid Chromatography. Elsevier, Amsterdam. 1985.

Kenner, C. T. Instrumental Separation Analysis. 1973.

Key, Blake D., Robert D. Howell, and Graig S. Criddle. *Fluorinated Organics in the Biosphere.* Environmental Science & Technology. Vol. 31. No.9. 1997.

Lindsay, W. L. *Chemical Equilibra in Soils.* Wiley Interscience, New York. 1979. pp. 374.

Locke, D.C. J. Chromatog. Sci. Vol. 12. 1974.

Martin, Paul, E. David Morgan, and Ian D. Wilson. *Effect of Carbon Loading on the Extraction Properties of C-18 Bonded Silica Used for Solid-Phase Extraction of Acidic and Basic Analytes.* Analytical Chemistry. Vol. 69. No. 15. Aug. 1, 1997. pp. 2972-2975.

Majors, R.R.. *High Performance Liquid Chromatography on Silica Gel.* Anal. Chem. 1972.

Mecray, J. G., E.A. Nowatzki, G. M. Thomson, D. J. Bestill, and R. Mitwasi. *Low Level Nuclear Waste Shallow Land Burial Trench Isolation.* National Technical Information Service, Springfield, VA. NUREG/CR-3084. 1983.

Melander, W.R. and Cs. Horvath. High-Performance Liquid Chromatography, Advances and Perspectives. Vol. 2. Academic Press. New York. 1980.

Meyer,Veronika R. Practical High-Performance Liquid Chromatography. 1993.

Parker, J. C. and M. Th. Van Genuchten. *Determining Transport Parameters from laoratory anf Field Tracer Experiments.* Va. Agric. Exp. Sta. Bull. Blacksburg, VA. 1984. pp.1794-1796.

Pearson, R.J., S.D. Comfort, and W.P. Inskeep. *Analysis of Fluorobenzoate Tracers by Ion Chromotograpgy.* Soil Sci. Soc. Am. J. 56. 1992. pp. 1794-1796.

Poole, C. F., S. Singhawangcha, L. E. Chen Hu, W. F. Sye. R. Brazell and A. Zlatkis. *Tert.-Butylpentafluorophenylmethylchlorosilane as a Reagent for the Formation of Hydrolytically Stable Alkylsilyl Derivatives with Electron-capturing properties.* J. Chromatogr. Vol. 187. 1980. pp. 331-340.

Poole, C. F., W. F. Sye, S. Singhawangcha, F. Hsu and A. Zlatkis. *New Electron-capturing Pentafluorophenyldialkylchlorosilanes as Versatile Derivatizing Reagents for Gas Chromatography.* J. Chromatogr. Vol. 199. 1980. pp. 123-142.

Reeve. R. N. J. Chromatography. Vol. 177. 1979. pp.393.

Sotomatatsu, T., M. Shigemura, and T. Fujita. *Correlation Analysis of the pKa values of Mono and Di-ortho-substituted benzoic Acids.* Bull. Chem. Soc. Jpn. Vol. 65. 1992. pp. 3157-3162.

Stetzenbach, K. J., S. L. Jensen, and G. M. Thompson. *Tracer Enrichment of Fluorinated organic Acids Used as Ground-Water Tracers by Liquid Chromatography.* Environ. Sci. Technol. Vol. 16. 1982. pp.250-254

Stetzenbach, K. J., and G. M. Thompson. *A New Method for Simultaneous Measurement of Cl^-, Br^-, NO_3^-, SCN^-, and I^- at sub-ppm Levels in Ground Water.* Ground Water. Vol. 21 1983.

Stetzenbach, K. J., Irene Farnham. *Organic Anionic Tracers: Chemistry and Toxicity.* 2nd Tracer Workshop, Univ. of Taxas at Austin. 1994.

Stetzenbach, K. J., Megumi Amano, David K. Kreamer, and Vernon F. Hordge. *Testing the Limits of ICP-MS: Determination of Trace Elements in Ground Water at the Part-Per-Trillion Level.* Ground Water. November-December 1994.

Strong, L. E., C. Van Waes, and K. H. Doolittle II. *Ionization of Five Aqueous Fluorobenzoic Acids: Conductance and Thermodynamics.* J. Solution Chem. V. II. 1982. pp. 237-258.

Thauer, J. R. and R. C. Huffacker. Analytical Biochemistry. Vol. 102. 1980. pp.110-116.

Verloop, A. and C. D. Ferrell. *Benzoylphenel Ureas - a New Group of Larvicides Interfering with Chitin Deposition.* Pesticide Chemistry in the 20th Century. ACS Symp. Series #37. 1977. p. 237

Wu, zhen. *Separation and identification of Difluorobenzoic Acids by HPLC/MS.* University of Nevada, Las Vegas. 1996.

Young, S. C. and J. M. Boggs. *Observed Migration of a Tracer Plume at the MADE Site.* Proc. Environmental Research Conf. on Groundwater Quality and Waste Disposal. Washington, DC. May 2-4, 1989. Electric Power Research Inst. Palo Alto, CA. EPRI EN-6749. 1990. pp.11-1 to 11-8.

VITA

Graduate College
University of Nevada. Las Vegas

Cong Han

Home Address:
2744 Los Claveles Apt. #1002
Providencia, Santiago
Chile

Degrees:

Bachelor of Science, Safety Engineering, 1990
Beijing Institute of Technology, Beijing, P.R. China

Master of Business Administration, Business Administration, 1995
City University, Seattle, WA

Special Honors and Awards:

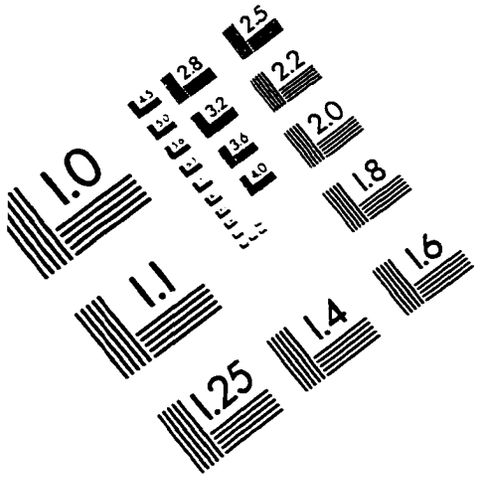
Graduate College Association Award, University of Nevada, Las Vegas

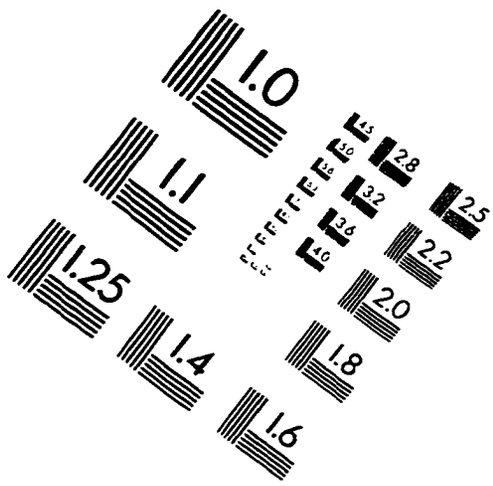
Thesis Title: Determination of Ground-Water Tracer 2,6-Difluorobenzoic Acid by GC/MS

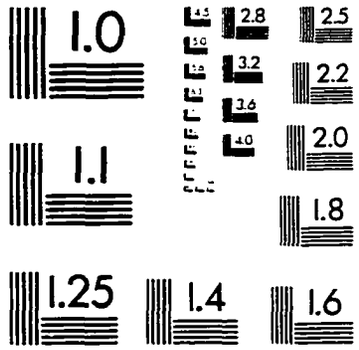
Thesis Examination Committee:

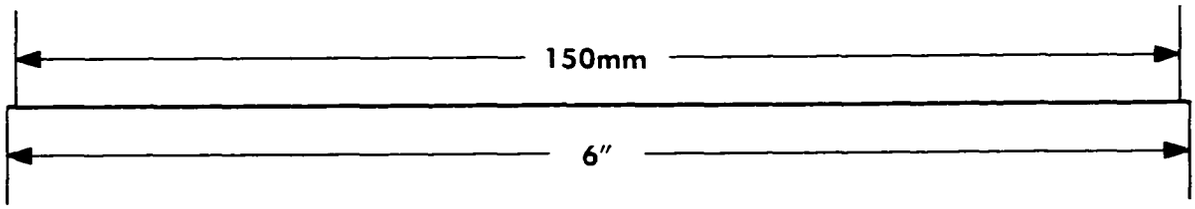
Chairperson, Dr. Klaus J. Stetzenbach, Ph.D.
Committee Member, Dr. Vernon F. Hodge, Ph.D.
Committee Member, Dr. Brian J. Johnson, Ph.D.
Graduate Faculty Representative, Dr. David E. James, Ph.D.

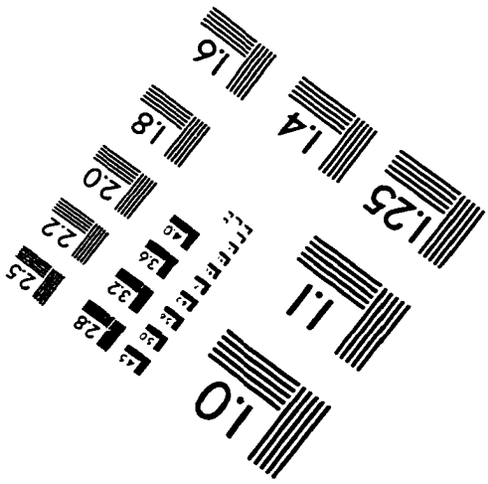
IMAGE EVALUATION TEST TARGET (QA-3)

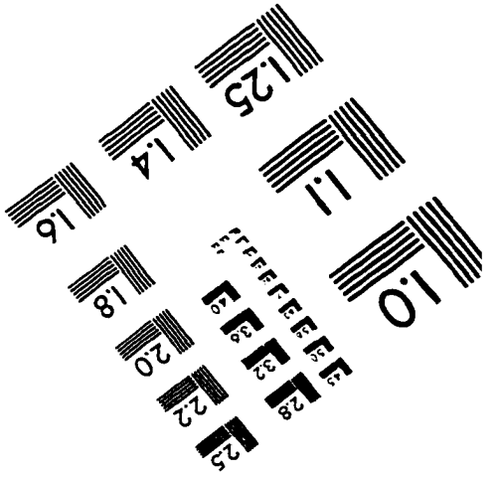












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989